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THE HOLOTHURINOGENINS

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Abstract—The chemistry of the holothurinogenins, derived from holothurin by acid hydrolysis, is discussed and structures for 22,25-oxido-holothurinogenin (Ia) and its 17-desoxy analog (IIa) are proposed.

MANY species of sea-cucumber in the family Holothuroidea of the phylum Echinodermata contain poisons for their defense against predators.[1] The isolation of the toxic principle named holothurin from the Caribbean species *Actinopyga agassizi* has been described.[1] Its various physiological activities have been the subject of a number of communications.[2] Holothurin (the separation of which into its components will be described in a separate communication) is a mixture of at least a half dozen glycosides, which yields on acid hydrolysis[1] four monosaccharides (xylose, glucose, 3-O-methylglucose and quinovose), sulfuric acid and a mixture of steroid aglycones closely related amongst each other. Two aglycones, 22,25-oxidoholothurinogenin (Ia) and its desoxy analog (IIa), have been obtained in pure form and comprise ca. 20 and 10% respectively of the total aglycone mixture.

In this paper, the structural investigations of these aglycones,[3] which have led to the formulations depicted below (Fig. 1) for 22,25-oxidoholothurinogenin (Ia) and its 17-desoxy derivative (IIa) are described. Of the remaining mixture of aglycones

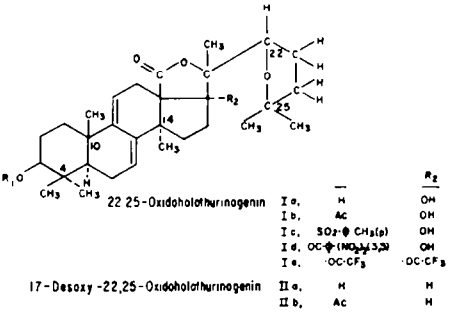

FIG. 1

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[1] J. D. Chanley, R. Ledeen, J. Wax, R. F. Nigrelli and H. Sobotka, *J. Amer. Chem. Soc.* **81**, 5180 (1959).

[2] S. L. Friess and R. C. Durant, *Toxicol. Appl. Pharmacol.* **7**, No. 3, 373 (1965); S. L. Friess, R. C. Durant, J. D. Chanley and T. Mezzetti, *Biochem. Pharmacol.* **14**, 1237 (1965), and Refs. cited therein.

[3] For preliminary communications on the structure of the holothurinogenins cf. J. D. Chanley and H. Sobotka, *The Holothurins, a Group of Animal Saponins.* Intern Symp. Org. Chem, of Natural Products, Ind. Chem. Belges, 532 (1962); H. Sobotka, *Chem. and Chem. Indy., Japan* **16**, 1023 (1963), H. Sobotka, *Bull. Soc. Chim. Biol.* **47**, no. 2, 169 (1965).

more than half is comprised of aglycones, provisionally designated holothurinogenin U (IIIa; Fig. 6), which have the same annular portion as Ia, but differ only in the structure of their side chains. The other aglycones are presumably related to the 17-desoxy series.

Nomenclature of the holothurinogenins. While compounds Ia and IIa comprise the largest individual fractions that have been isolated in pure crystalline form, it is preferable to apply the term holothurinogenin to the open chain analog. This is not only expedient for purposes of nomenclature, but also takes into account that the greater part of the aglycones may have open side chains; in fact, the possibility may not be ignored that the oxide ring in the side chain is formed, during the acid hydrolysis, from those glycosides that combine an hydroxyl group on C-22 with a double bond or hydroxyl issuing from C-25. The closure of an oxide ring in a similar situation under acid conditions has been reported by Shibata[4] for the hydrolysis of Ginseng saponin to panaxadiol. Holothurinogenin is then 3β,17α,20ξ-trihydroxy-5α-lanosta-7:8,9:11-diene-18-carboxylic acid lactone (18 $\rightarrow$ 20). The names of the various congeners such as the 17-desoxy series, the 22,25 oxido-compounds and others can be derived from this parent substance.

22,25-*Oxidoholothurinogenin* (Ia). The elementary analysis of Ia, m.p. 315–316°, $[\alpha]_D^{25}$ −21·2°, as well as a considerable number of its derivatives and the observed molecular ion peaks, *m/e*, 484 and 482 for it and the derived monoketone (IV) respectively established the molecular formula $C_{30}H_{44}O_5$.

Double bonds. That 22,25-oxidoholothurinogenin (Ia) contains a *trans* hetero-annular conjugated diene system, in which each of the double bonds is triply substituted, was deduced from the following observations. In the UV, Ia exhibits a triple absorption band, characteristic of an heteroannular diene,[5] with the maximum at 243 mμ (ε 14,400) and two prominent shoulders (sh) at 237 mμ (ε 13,430) and 252 mμ (ε 10,390). Its acetate (Ib), shows in the IR an absorption band at 814 cm^{-1}, comparable in position and intensity with that ascribed to the vinylic hydrogens of 24,25-dihydroagnosteryl acetate.[6] The NMR spectrum of the acetate (Ib) shows two broad signals at 5·51 and 5·25 ppm, each with an intensity corresponding to one vinylic hydrogen. The acetate (Ib) was recovered after refluxing with maleic anhydride, in xylene, for several hr. It should be noted at this point that the glycosides from which the aglycone (Ia) is derived show no significant UV absorption above 212 mμ. The diene system is formed in the acid hydrolysis of the glycoside mixture.

Oxygen functions. Of the five oxygen functions present two are accounted for in hydroxyl groups, one secondary, the other tertiary; two are contained in a five membered ring lactone; while the fifth is an ether oxygen. The nature of the oxygen functions is based on the following observations.

22,25-Oxidoholothurinogenin (Ia) forms a monoacetate, $C_{32}H_{46}O_6$ (Ib), m.p. 289–290°, $[\alpha]_D^{25}$ 6·5°; a mono-*p*-toluenesulfonate (Ic), m.p. 170–171° and a mono-3,5-dinitrobenzoate (Id), m.p. 301°. Each of these derivatives still retains an hydroxyl group as demonstrated by their IR absorption at ca. 3565 cm^{-1} (CCl_4). Oxidation of

[4] S. Shibata, O. Tanaka, M. Sado and S. Tsushima, *Tetrahedron Letters* No. 12, 795 (1963).

[5] E. R. H. Jones and T. G. Halsall, *Progress in the Chemistry of Organic Natural Products* (Edited by L. Zechmeister) Vol. XII; pp. 82, 104. Springer Verlag, Wien (1955).

[6] L. J. Bellamy, *The Infrared Spectra of Complex Molecules* p. 44. Wiley, New York (1954).

Ia with chromic oxide–pyridine mixture yields the monoketone 22,25-oxidoholothurinogenone (IV), $C_{30}H_{42}O_5$, m.p. 296–297°, $[\alpha]_D^{25}$ −41·6° (Fig. 2). The IR (ν_{max} (CCl_4) 3567 (OH) and 1709 cm^{-1} (C=O)) and UV (λ_{max} 244 mμ (ε 14,400) λ_{sh} 237 mμ (ε 13,430), λ_{sh} 252 (ε 10,950)) spectra of the ketone show the carbonyl to be in a six membered ring, not conjugated with the diene system, and the presence of the non-acylable hydroxyl group. 22,25-Oxidoholothurinogenin (Ia) yields under forcing conditions a bistrifluoroacetate (Ie), $C_{34}H_{42}O_7F_6$, m.p. 260–261°, the IR spectrum of which no longer contains an hydroxyl grouping. The secondary nature of one of the hydroxyls is thus established, while the inferred tertiary character of the other was confirmed by degradative experiments to be described.

The presence of a hindered five membered ring lactone in Ia is indicated by the strong absorption band in the IR at 1763 cm^{-1} ($CHCl_3$), and the corresponding lactone carbonyl absorption of its acetate (Ib) at 1773 (CS_2) and 1767 cm^{-1} ($CHCl_3$). The aglycone (Ia) was recovered unchanged after prolonged refluxing with alcoholic KOH (10%).

The ethereal character of the fifth oxygen was thus indicated by exclusion. Further evidence for both the nature of this oxygen as well as its immediate environment can be deduced from the IR and NMR spectra. 22,25-Oxidoholothurinogenin (Ia) and its acetate (Ib) each show a strong absorption band attributable to an ether linkage, at 1134 cm^{-1} ($CHCl_3$ and/or CS_2). The NMR spectrum of the acetate (Ib) reveals the presence of two tertiary hydrogens bound to oxygen bearing carbon atoms. One of these signals appearing at 4·56 ppm (broad) may be ascribed to the hydrogen bound to the carbon atom bearing the acetyl group; the other, a broad triplet, centered at 4·23 ppm, J = 6 c/s, indicates a methine hydrogen, whose α neighbors are the ether oxygen and a single methylene group. A tri-substituted (α,α,α') cyclic ether can reasonably accommodate the observed NMR.

Methyl groups. The NMR spectra of 22,25-oxidoholothurinogenin acetate (Ib) reveals the presence of seven methyl groups attached to quaternary carbon atoms. In $CDCl_3$ the resonance signals appear at 0·91, 0·99, 1·15, 1·22, 1·38, each of an intensity corresponding to three hydrogens, and at 1·26 and 1·28 ppm (not completely resolved) representing six hydrogens. In pyridine resolution is achieved and the seven equivalent signals appear at 0·90, 1·02, 1·08, 1·15, 1·37, 1·50 and 1·59 ppm.

Of the naturally occurring thirty carbon atom compounds the tetracyclic, but not the pentacyclic triterpenes can "a priori" accommodate a $C_{30}H_{44}O_5$ compound, which contains seven quaternary methyls, two double bonds in conjugation, one ether and one lactone ring. On the assumption that 22,25-oxidoholothurinogenin (Ia) is a tetracyclic triterpene, the following conclusions and reasonable conjectures may be made with regard to its structure. (1) The triterpenes belonging to the dammaranes or isoeuphanes were excluded from consideration, since they can not accommodate a *trans* heteroannular diene, in which each of the double bonds is trisubstituted. (2) The presence of a lactone ring and only seven methyl groups indicates that one of the eight methyl groups anticipated for a triterpene is oxidized to an acid (lactone). (3) The multiplicity and position of the methyl resonance signals (1·38, 1·28, 1·26, and 1·22 ppm) indicate that the three methyl groups, C-21, C-26, C-27, of the triterpene side chain are attached to oxygen bearing carbon atoms. The position of the furthest

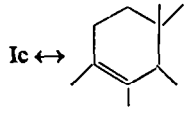
downfield signal, 1·38 ppm (3H), suggests[7] that one methyl group, C-21, is bound to the carbon atom bearing the lactonic oxygen. (4) The oxidized methyl group is the C-18 methyl and the five membered ring lactine terminates on C-20. This conclusion is based on the following considerations. The stability of the lactone to alkali makes it unlikely that it originates from any methyl group other than those attached to C-13 or C-14. Furthermore, if the lactone is derived from any methyl group other than C-18, a methyl signal attributable to C-18 would appear at substantially higher field[8,9] than observed (0·90 ppm). In fact, both 24,25-dihydroagnosteryl and 24,25-dihydrolanosteryl acetates show methyl signals at 0·70 and 0·49 ppm, respectively.[10] (5) The disposition of the ether oxygen to the side chain C_{22}–O–C_{25}, appears most reasonable, since it accounts for the stability of the ether to acid, the downfield displacement of the C-26 and C-27 methyl signals and contains a methine hydrogen whose adjacent α-neighbors are an oxygen atom and a single methylene group. (6) In accord with the UV, IR, and NMR spectra, therefore, the diene can occupy positions 7:8, 9:11 in rings B and C, or 5:6, 10:1 in rings A and B. For the first case 22,25-oxidoholothurinogenin (Ia) belongs to the lanostane or euphane triterpenoids and for the alternative disposition of double bonds (5:6, 10:1) to the cucurbitacins.

The proposed structure (Fig. 1) for 22,25-oxidoholothurinogenin (Ia) is based on the following additional findings.

3β-Hydroxy-4,4-gem-dimethyl. The presence and similar disposition of a *gem*-dimethyl and adjacent secondary hydroxyl group in 22,25-oxidoholothurinogenin and the triterpenes is indicated by the appearance of a sharp band of medium intensity at 1389–1390 cm^{-1} (CCl_4) for both its acetate and those of 24,25-dihydrolanosterol and agnosterol. The corresponding alcohols and ketones exhibit only inflections (if at all) in this region. The question as to whether this absorption band, as in the case of steroid acetates, should be ascribed to the higher frequency member of the split acetyl methyl absorption,[11] the *gem*-dimethyl grouping,[12] or both is not germane to the argument. In any case, for both steroid and various sapogenin acetates examined, the higher frequency of the split acetyl methyl absorption appears generally as a weak inflection at significantly lower frequencies (1375–1382 cm^{-1}). The *gem*-dimethyl and proximate hydroxyl groups in Ia was demonstrated in a manner analogous to that employed[13] with various triterpenes. The *p*-toluenesulfonate, compound (Ic) on heating in acetic acid loses a molecule of *p*-toluenesulfonic acid[14–16] and yields a mixture of trienes (V) (Fig. 2). The triene mixture in which the three double bonds are not linearly conjugated (UV) exhibits no methyl signal (NMR) at higher field than 1·25 ($CDCl_3$). While the region of methyl resonance signals is too complex for complete analysis, the appearance of a strong signal at 1·7 ppm (3–6H) and absence

[7] D. H. R. Barton, H. T. Cheung, P. J. L. Daniels, K. G. Lewis and J. F. McGhie, *J. Chem. Soc.* 5163 (1962).
[8] D. Lavie, Y. Shvo and E. Glotter, *Tetrahedron* **19**, 2255 (1963).
[9] R. F. Zürcher, *Helv. Chim. Acta* **46**, 2054 (1963); *Ibid.* **44**, 1380 (1961).
[10] Unpublished observations, cf. L. B. Kier and W. S. Brey Jr., *J. Pharm. Sci.* **52**, 5 (1963).
[11] R. N. Jones and A. R. H. Cole, *J. Amer. Chem. Soc.* **74**, 5648 (1952).
[12] D. H. R. Barton, J. E. Page and E. W. Warnhoff, *J. Chem. Soc.* 2715 (1954).
[13] For numerous examples cf. J. L. Simonsen and W. C. J. Ross, *The Terpenes*, Vols. IV and V (esp. Vol. IV, pp. 39–55, 130–131) Cambridge University Press (1957).

of the methyl peaks at 0·90 and 0·99 ppm clearly indicates that two methyl groups are involved in the reaction and a rearrangement has occurred. Hydroxylation of the triene mixture (V) with osmium tetroxide, and oxidation of the derived crude glycol mixture with lead tetraacetate yields acetone and formaldehyde (Fig. 2). Although acetone is obtained in smaller yield than formaldehyde, the occurrence of both products is best accounted for by a retropinacolinic rearrangement of the *p*-toluenesulfonate (Ic) with concomitant significant migration of the newly formed double bond from the isopropylidene to the isopropylene position. An alternate explanation for the aforementioned finding, namely, that a simple migration of one of the methyl groups of the *gem*-dimethyl has occurred was rejected, since it not only can not



account for the formation of acetone, but necessitates the assumption[14–17] that either the junction of rings A and B is *cis* and the hydroxyl β (axial) or that with the junction *trans* the hydroxyl group is α (axial). Optical rotational data (discussed below) contradicts both these assumptions, but are in full accord with the stereochemistry: 3β-hydroxy, *trans*-A/B, as required for a retropinacolinic rearrangement.

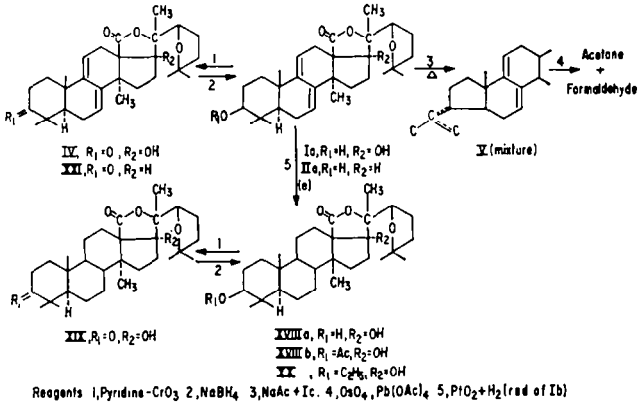


FIG. 2.

Cleavage of 22,25-*oxidoholothurinogenin* (Ia) *to the* C_8 *ketone*, (VII) *and* C_{23} *ketone*, (VIII). 22,25-Oxidoholothurinogenin acetate (Ib) on reduction with LAH gives the tetrol (VIa) which on acetylation furnishes the diol-diacetate, VIb ($C_{34}H_{52}O_7$). Their formulation, as shown in Fig. 3, is consonant with the following observations. The crude tetrol (VIa) shows no carbonyl absorption in the IR. The diol-diacetate (VIb) exhibits in the UV the typical triple peak of an heteroannular diene system with

[14] G. Bancroft, Y. M. Y. Haddad and G. H. R. Summers, *J. Chem. Soc.* 3295 (1961); Y. M. Y. Haddad and G. H. R. Summers, *Ibid.* 769 (1959).

[15] C. W. Shoppee and G. A. R. Johnston, *J. Chem. Soc.* 3261 (1961).

[16] The same product (mixture), but in significantly smaller yield and accompanied by considerable amounts of chlorinated by products was obtained by the action of PCl_5 on Ia in either pet. ether or benzene and under N_2. A similar experience has been reported in the rearrangement of an eburicoic acid derivative; cf. J. Fried and E. F. Sabo, *J. Amer. Chem. Soc.* **84**, 4356 (1962).

[17] D. H. R. Barton, *J. Chem. Soc.* 1027 (1953).

maxima at 238 mμ (ε 14,900), 243 mμ (ε 17,250) and 252 mμ (ε 11,370). The NMR spectrum of VIb shows signals attributable (a) to seven tertiary methyls (0·88 (3H), 0·95 (3H), 1·01 (3H), 1·25 (6H), 1·34 (3H), 1·38 (3H) ppm); (b) to two vinyl hydrogens, 5·44 ppm (broad), (c) to two tertiary hydrogens on oxygen bearing carbon atoms, 4·56 ppm (1H) (broad) and 4·23 ppm (1H), J = 6 c/s (broad triplet), the first and second referring to the hydrogens on carbon atoms C-3 and C-22 respectively. The appearance of a pair of doublets centered at 3·86 (1H) and 4·30 ppm (1H) with a coupling constant of J = 12 c/s confirms[18] the presence of a tertiary oxymethylene group with two non-equivalent hydrogens.

Oxidation of the diol diacetate (VIb) with lead tetraacetate results in cleavage of the molecule without loss of carbon into two ketones, one with twenty two carbon atoms (VIIIa) and a volatile one with eight carbon atoms (VII). This demonstrates that the lactone extends from the annular portion of the molecule to the side chain and indicates that the non-acylable hydroxyl group, also present in 22,25-oxidoholothurinogenin, is on the β carbon (C-17) atom of the lactone ring.

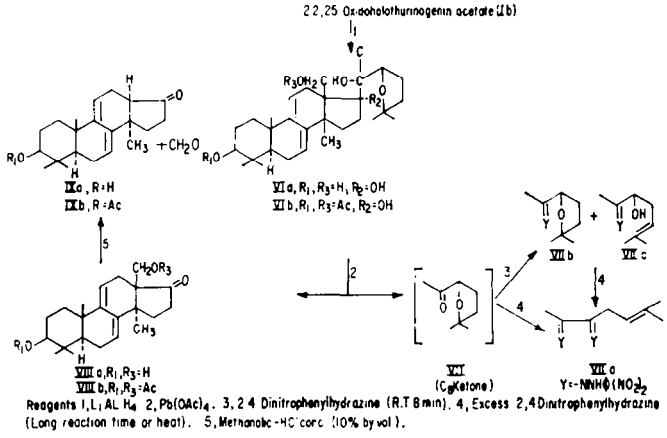


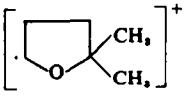
FIG. 3

The C_8 *ketone,* (VII). The volatile fragment yields, depending on reaction conditions, a single osazone or a mixture of 2,4-dinitrophenylhydrazones (Fig. 3). Protracted treatment (12 hr room temp) or heating of the volatile fragment with excess Brady's reagent results in the formation of the open chain osazone (VIIa) of 6-methylhept-5-ene-2,3-dione. The osazone (VIIa) is identical in all respects, analysis, m.p., UV and IR with an authentic sample. The osazone was synthesized by treating the crude reaction product, derived from the action of nitrous acid on 6-methylhept-5-ene-2-one, with excess Brady's reagent. Shorter reaction time (8 min) of the volatile ketone and reagent and immediate extraction of the mixture with Skelly B gives a mixture of two 2,4 DNP derivatives, which may be separated by crystallization and chromatography. One of the derivatives, m.p. 129–130°, shows the presence of an OH group (IR). The other, an oil, shows no OH. Both derivatives are unconjugated (UV) and each furnishes the same osazone (VIIa) on prolonged reaction or heating with excess reagent. While neither derivative was obtained in sufficient quantities for analysis,

[18] W. T. de Kock, P. R. Enslin, K. B. Norton, D. H. R. Barton, B. Sklarz and A. A. Bothner-By *J. Chem. Soc.* 3828 (1963); T. Takahashi, *Tetrahedron Letters* 565 (1964).

it is reasonable to assume that the one of m.p. 130° is the hydrazone (VIIc) of the open chain 3-hydroxy-6-methylhept-5-ene-2-one, while the other (VIIb) retains the ether linkage.

The presence of the cyclic ether on the side chain of 22,25-oxidoholothurinogenin is thereby established and further confirmation was obtained from mass spectroscopy. The aglycone (Ia) and the derived ketone (IV) each give strong ion peaks at *m/e* 99, corresponding to the cyclic ether fragment.



The C_{22} *ketone*, (VIIIa). The larger ketonic fragment (VIIIa) after acetylation gives the keto-diacetate, $C_{28}H_{36}O_5$, formulated as VIIIb (Fig. 3). The latter compound exhibits a molecular ion peak, *m/e*, 428 confirming the empirical formula. Compound VIIIb has retained the parent diene system, λ_{max} 242 mμ (ε 16,600), λ_{sh} 236 mμ (ε 15,100), λ_{sh} 252 mμ (ε 10,000). It may be shown by IR to be devoid of a free hydroxyl group, while the carbonyl group is in a five membered ring and flanked by one methylene group. (ν_{max}(CCl_4) 1740 and 1410 cm^{-1} (CH_2CO)). The NMR spectrum of VIIIb shows the presence of four quaternary methyl groups, 0·90 (6H), 0·98 (3H) and 1·06 ppm (3H), each attached to saturated non-oxygen bearing carbon atoms; two vinyl hydrogens centered at 5·50 (1H) (broad) and 5·70 (1H) (broad); a pair of doublets centered at 3·70 (1H) and 4·33 (1H) ppm, J = 12 c/s, (CH_2OCOCH_3), and only one hydrogen bound to an oxygen bearing carbon atom, 5·43 ppm, related to the secondary acetyl group. Compound VIIIb on mild hydrolysis (methanolic HCl, 10%, room temp) gives the crude parent dihydroxy ketone (VIIIa) which exhibits the same UV as the diacetate (VIIIb) while its carbonyl absorption appears at 1735 cm^{-1}. These results clearly demonstrate that the side chain of compounds VIIIa,b and therefore 22,25-oxidoholothurinogenin, is attached to carbon atom 17 and the tertiary hydroxyl (non-acylable) of 22,25-oxidoholothurinogenin occupies the 17 position. (Fig. 3)

The supposition (*vide supra*) that the five membered ring lactone extends from C-18 to C-20 was confirmed by the following. The C_{22} ketone (VIIIb) on refluxing under nitrogen with methanolic HCl gives one equivalent of formaldehyde. This retroaldolization[19] would be anticipated for a crutch oxymethylene grouping placed α to a carbonyl. The desoxymethylene ketone (IXa) gives a monoacetate (IXb; $C_{23}H_{32}O_3$) and is formulated as the C/D *cis* derivative based on ORD measurements discussed below. (Fig. 3). Compound IXb exhibits a simple broad maximum in the UV at 236 mμ (ε 10,900), while its IR spectrum confirms the presence of the five membered ring ketone (ν_{max} 1744 and 1402 cm^{-1}) and absence of an hydroxyl group. The NMR spectrum of IXb confirms the presence of four quaternary methyl groups (0·86 (6H), 0·95 (3H) and 1·32 ppm (3H) ($CDCl_3$)). In benzene the resolution of the methyl signals was achieved with equivalent absorption at 0·72, 0·77, 0·88, 1·12 ppm. The position and low intensity of the UV maximum raises the question as to whether or not the original heteroannular diene system is still present. This was established by the NMR spectrum and a comparison of its IR spectrum with those of agnosteryl

[19] Refluxing of VIIIb with alcoholic KOH (5–10%) also gave in small yield of IXa.

acetate and the keto-diacetate (VIIIb). Compound IXb exhibits in the IR absorption at 3033 (CCl_4), 817, 798 cm^{-1} (CS_2) indicative of vinyl hydrogens attached to a trisubstituted double bond. The intensity and positions of these bands are comparable to that of 24,25-dihydroagnosteryl acetate (3033 (CCl_4), 812, 792 cm^{-1}) and the keto-diacetate, VIIIb, (3033 (CCl_4), 812, 798 cm^{-1}). The NMR spectrum of VIIIb shows the presence of 2 vinyl hydrogens, whose signals are centered at 5·50 (1H) (broad) and 5·90 ppm (1H) (broad).

Double bonds (7:8, 9:11). The proposed disposition (7:8, 9:11) of the conjugated bond system was confirmed by the following findings. The LAH reduction product (VIa or VIb) on treatment with methanolic HCl yields a non-conjugated monoene (Xa; $C_{30}H_{48}O_5$), m.p. 264–265°. This compound shows no significant UV absorption above 210 mμ, contains one trisubstituted double bond (807 cm^{-1}, $\diagdown C{=}CH\diagup$) and yields only a monoacetate (Xb), $C_{32}H_{50}O_6$, m.p. 248°. Oxidation of Xa with lead tetraacetate gives the large ketonic fragment (XIa; $C_{22}H_{32}O_3$) m.p. 200–201°, whose IR demonstrates the presence of a five membered ring ketone (1736 cm^{-1}) and a new ether linkage (1097 cm^{-1}). Compound XIa gives only a mono-acetate (XIb; $C_{24}H_{34}O_4$), m.p. 181–182°, whose IR demonstrates the absence of an hydroxyl group. Each of the aforementioned products (X and XI) therefore contains an ether linkage which undoubtedly is originally formed by the action of methanolic HCl on the LAH reduction product (VIa). The loss of one acylable hydroxyl (on C-18), conjugation, and concomitant formation of a new ether (VI → X) clearly places the ether oxygen between C-11 and C-18. (Fig. 4). The formation of the new ether is readily understood in terms of mechanisms involving either addition of a proton to the 9:11 double bond or alternatively addition of the proton to the 7:8 double bond and migration of the double bond to the 8:9 position (ultimatley to the 7:8 position) and attack of the oxymethylene group (—CH_2OH) on the positively charged carbon atom 11.

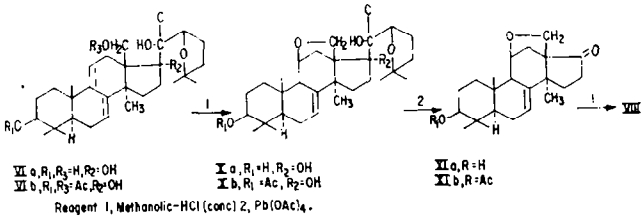


FIG. 4

The double bond system, 7:8, 9:11, in 22,25-oxidoholothurinogenin is corroborated by the following findings. Oxidation of the acetate (Ib) with chromic oxide yields the yellow *trans* ene-dione (XII; $C_{32}H_{44}O_8$), m.p. 218–219°, λ_{max} 280 mu (ε 6,390) (Fig. 5). Ene-diones are typical products[20] of oxidation of tetracyclic triterpene, 7:8, 9:11, dienes. In addition the conjugated monoketone, XIIIb, (7 keto-8-ene), $C_{32}H_{46}O_7$, m.p. 262–263°, λ_{max} 255·5 mμ (ε 10,050) was obtained. Compound XIIIb forms a 2,4-dinitrophenylhydrazone derivative with UV absorption, λ_{max} 393 mμ (ε 22,400) typical of those derived from conjugated ketones. Compound XIIIb was prepared by two additional routes. (Fig. 5) The crude oxide mixture obtained by the action of perbenzoic acid on 22,25-oxidoholothurinogenin acetate

[20] Cf. Ref. 6, p. 54.

(Ib), yields after treatment with BF_3-etherate[21] the compound XIIIb. 22,25-Oxidoholothurinogenin acetate in pyridine, reacts with osmium tetroxide to give XVa, $C_{32}H_{48}O_8$, formulated as the 7:8 diol for the following reasons (Fig. 5). The diol (XVa) does not show significant absorption above 210 mμ and gives on acetylation a diacetate (XVb, $C_{34}H_{50}O_9$). Compound XVa on standing with methanolic HCl and subsequent reacetylation of the crude reaction product yields the "7-keto-8:9-ene", (XIIIb). The initial product of acid treatment is the unacetylated "ene-one" (XIIIa) since it gives no acetyl band in the IR and shows a maximum in the UV at 256 mμ (ε 8,300). Oxidation of XIIIa with chromic oxide–pyridine mixture affords the corresponding 3-keto derivative (XIV) whose UV absorption is unaffected by the addition of alkali.[22]

The 7:8 diol (XVa) on oxidation with lead tetraacetate is cleaved to the conjugated keto aldehyde (XVI; $C_{32}H_{46}O_8$). Consonant with this formulation are the observed absorption bands in the UV, λ_{max} 233·5 mμ (ε 7,060), and IR, ν_{max} (CCl_4) 2731 (C*H*O), 1772 (lactone), 1740–1729 (aldehyde + acetate) and 1681 cm^{-1} (conjugated ketone).

In addition to the aforementioned 7:8 diol (XVa) a second unsaturated ketone (XVII; $C_{32}H_{46}O_7$), m.p. 331–332°, λ_{max} 265 mμ (ϵ 7,500) is obtained directly from the decomposition of the osmate ester. This ketone does not form a 2,4-dinitrophenylhydrazone and is tentatively formulated as the 11 keto derivative. The relative proportions of this ketone (XVII) to the diol (XVa) varied considerably for different runs and in some instances XVII was the major product. The factors governing this variation have not been ascertained.

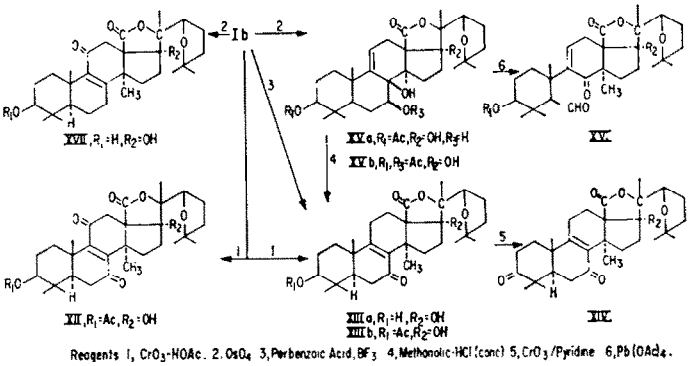
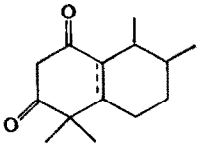


FIG. 5

[21] H. Heusser, R. Eichenberger, P. Kurath, H. R. Dällenbach and O. Jeger, *Helv. Chim. Acta* **34**, 2106 (1951).

[22] The fact that the spectrum of XIV was unaffected by alkali almost certainly precluded it being an α,β-unsaturated 1,3-diketone e.g. (cf. D. H. R. Barton, P. J. L. Daniels, J. F. McGhie and P. J.



Palmer, *J. Chem. Soc.* 3675 (1963)) and therefore makes it unlikely that 22,25-oxidoholothurinogenin (Ia) contained the diene 5:6, 10:1 (cucurbitacin cf. text), from which a 1:3-diketone derivative may be anticipated.

It was shown that in acid solution an equilibrium exists between VIIIa ↔ XIa and VIa ↔ Xa, which lies in favor of the conjugated dienes VIIIa and VIa respectively. A study of the equilibrium between these pairs, in methanolic HCl (10%) at 37°, was made by noting the change in absorbance with time at several wavelengths; 230, 242 and 250 mμ. Equimolar concentrations of either VIIIa or XIa gives after ca. 4 days the same constant optical density readings at each of the wavelengths, indicating that equilibrium is attained. A 90:10 mixture in favor of the conjugated diene (VIIIa) was observed. Similarly, Xa gives an equilibrium mixture containing ⩾95% of VIa. The isolation of the unconjugated Xa derives from its extreme insolubility and consequent precipitation from the concentrated reaction mixture (cf. above). Acid treatment of VIIIa does not yield XIa in isolable amounts.

22,25-Oxidoholothurinogenin acetate dissolved in acetic acid, containing a few drops of $HClO_4$, is reduced catalytically (PtO_2) to the perhydroderivative (XVIIIb, $C_{32}H_{50}O_6$), m.p. 267–268°, $[\alpha]_D^{25}$ −9°. (Fig. 2). The reduction, as in the case of agnosterol acetate,[23] proceeds slowly and it is necessary to employ excessive amounts of catalyst. The saturated derivative is obtained from the complex mixture of reduction products after extensive chromatography and recrystallization. The saturated derivative (XVIIIb) shows no absorption in the UV (209 mμ, ε 280) and its IR absorption confirms the presence of the five membered ring lactone (1760), side chain ether (1131, 1112) and tertiary hydroxyl (3556 cm^{-1}) groups. On alkaline hydrolysis it gives the saturated aglycone (XVIIIa, $C_{30}H_{48}O_5$), m.p. 321–322°, $[\alpha]_D^{25}$ −19·9°, which on oxidation with chromic oxide–pyridine mixture furnishes the saturated ketone (XIX, $C_{30}H_{46}O_5$), m.p. 310°, $[\alpha]_D^{25}$ −34·2°, IR ($CHCl_3$) 3560 (OH), 1751 (lactone), 1695 cm^{-1} (six membered ring ketone). In analogy with the findings[23] obtained in the hydrogenation of agnosteryl acetate, the hydrogens C-8 and C-9 are assigned the configurations β and α respectively. A second minor reduction product (XX) whose physical properties are in agreement with its formulation as the 3β-ethyl ether of 22,25-oxidoholothurinogenin, was isolated. Compound XX, $C_{32}H_{52}O_5$, m.p. 253–254°, $[\alpha]_D^{25}$ +3·4° shows no significant absorption in the UV (209 mμ, ε 280) and in the IR (CS_2) it gives in addition to absorption bands for the tertiary hydroxyl (3556), five membered lactone (1761), side chain ether (1131, 869), also a strong absorption at 1104 cm^{-1} attributable to the —OC_2H_5 grouping. No carbonyl absorption for an acetyl group was observed.

17-*Desoxy*-22,25-*oxidoholothurinogenin* (IIa). Elementary analysis of IIa, m.p. 286°, $[\alpha]_D^{25}$ −9·3° as well as a few of its derivatives established the empirical formula $C_{30}H_{44}O_4$. In the UV the compound exhibits the typical triple absorption for an heteroannular diene; λ_{max} 242 (ε 16,800), λ_{sh} 237 (ε 15,000), λ_{sh} 252 mμ (ε 11,800). IR absorption bands attributable to an OH (3632), five membered lactone (1771), side chain ether (1132, 1117 cm^{-1}) groups are observed. The desoxy compound on acetylation yields a monoacetate, (IIb, $C_{32}H_{46}O_5$), m.p. 266·2°, $[\alpha]_D^{25}$ +21·3° and on oxidation with chromic oxide pyridine mixture, the monoketone (XXI; $C_{32}H_{42}O_4$), m.p. 282–283°, $[\alpha]_D^{25}$ −24·8°. The IR spectrum of the acetate IIb and the ketone XXI (1709 cm^{-1}, six membered ring ketone) show them each to be devoid of an OH-group, while the absorption for the lactone, side chain ether and diene (UV) are undisturbed. The NMR spectra of 17-desoxy 22,25-oxidoholothurinogenin acetate

[23] J. D. Chanley and T. Mezzetti, *J. Org. Chem.* **29**, 228 (1964).

(IIb) reveals the presence of seven quaternary methyl groups (1·35, 1·26, 1·22, 1·13, 1·00, 0·96 and 0·90 ppm), two vinyl hydrogens (5·58 and 5·29 ppm) (broad), two tertiary hydrogens bound to oxygen bearing carbons: 4·56 (br.) (C_3—H) and 4·23 ppm (C_{22}—H). The parallelism between the physical and chemical properties of IIa and 22,25-oxidoholothurinogenin leaves no doubt as to its formulation as the 17-desoxy-derivative of the latter. (Fig. 1).

Holothurinogenin U. In analogy to 22,25-oxidoholothurinogenin, the holothurinogenin U mixture (Experimental) was reduced with LAH, the crude reduction product reacetylated and cleaved with lead tetraacetate into non-volatile and volatile ketonic fragments (Fig. 6). The non-volatile ketonic fragment gives in relatively high

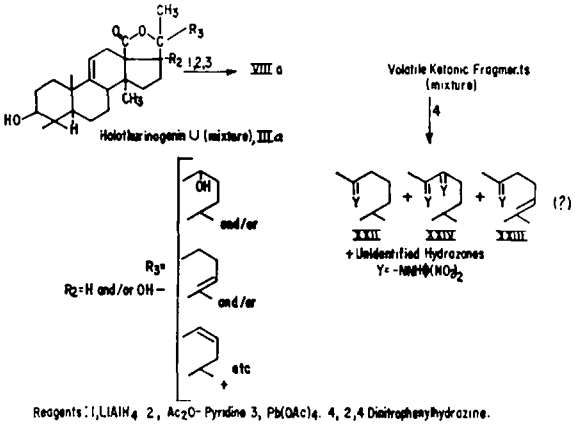


FIG. 6

yield, the C_{22} ketone (VIIIb) identical in all respects, m.p., IR, UV, with that derived from 22,25-oxidoholothurinogenin (Ia). The volatile material was a complex mixture of saturated and unsaturated ketones, from which it was possible to isolate in poor yield the 2,4-dinitrophenyl hydrazones of 6-methylheptan-2-one (XXII) and (?) 6-methylhept-5-ene-2-one (XXIII), and the osazone of 6-methylheptan-2,3-dione (XXIV). The first and last derivatives are identical in all respects, m.p., IR, UV, with authentic samples.

Stereochemistry of 22,25-*Oxidoholothurinogenin.*

3β-OH- *and* A/B-trans-(10β, 5α). The ORD curves of 22,25-oxidoholothurinogenone (IV), and perhydro-22,25-oxidoholothurinogenone (XIX) (Fig. 7) both show a negative Cotton effect analogous to those found for various 3-keto tetracyclic type triterpenes[24] of known, 10β, 5α configuration. Of particular significance is the fact that, whereas a negative Cotton effect is observed with the saturated tetracyclic triterpenes, only those unsaturated compounds which contain a double bond in the 7:8, or the conjugated diene system 7:8, 9:11 behave similarly.[25] It has been reported[26] that 4,4-dimethyl-5β-cholestan-3-one (10β, 5β) also shows a negative Cotton effect. The possibility that 22,25-oxidoholothurinogenin has this configuration

[24] C. Djerassi, *Optical Rotary Dispersion* pp. 89–102. McGraw-Hill, New York (1960).
[25] C. Djerassi, O. Halpern, V. Halpern and B. Riniker, *J. Amer. Chem. Soc.* **80**, 4001 (1958).
[26] G. R. Chaudry, T. G. Halsall and E. R. H. Jones, *J. Chem. Soc.* 2725 (1961).

was excluded by the following findings. The ORD curve of XIIIb, "7-keto-8:9 ene", (Fig. 7) shows a negative multiple Cotton effect, completely analogous to that reported[25] for 3β-benzyloxy-5α-lanost-8-ene-7-one and 3β-acetoxy-5α-cholest-8-ene-7-one. The ORD curve of methyl-3α-acetoxy-7-ketochol-8-eneate,[27] on the other hand, is very nearly the mirror image of the aforementioned compounds. The stereochemical designation β-equatorial for the 3-hydroxyl group then follows from the fact that, as with all triterpenes[28] of 3β-OH, 10β, 5α configuration, the rotation of 22,25-oxidoholothurinogenin acetate, (as well as with its various derivatives) is more dextrorotatory, $[\Delta M]_D = +131°$, than the parent alcohol. The 3-axial acetates

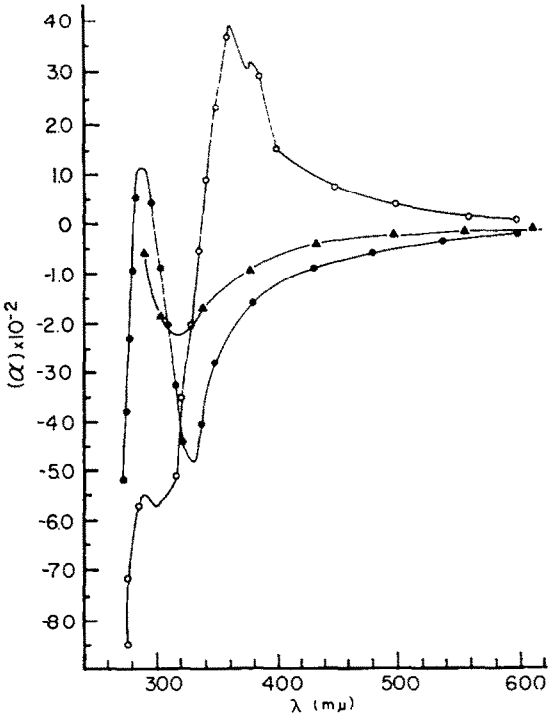


FIG. 7. ORD curves of IV —●—●—, XIX —▲—▲—, and XIIIb —o—o—o in dioxane.

(3α, 10β-5α) of tetracyclic triterpenes are less dextrarotatory than their parent alcohols. The reduction by sodium boronhydride[14,29] of both, 22,25-oxidoholothurinogenone (IV) and its perhydro derivative (XIX) to the original aglycones Ia and XVIII respectively provides further confirmation for these assignments. These results independently corroborate the assignment of a *gem*-dimethyl group at C-4 and confirm the stereochemical assignments based on the retropinacolinic rearrangement and IR data (vide supra).

13β, 14α *Configuration.* The configuration, 13β, 14α, for the C-18 (lactone) and the methyl group at C-14 respectively, suggested by the position[5] of the UV absorption

[27] C. Djerassi, R. Riniker and B. Riniker, *J. Amer. Chem. Soc.* **78**, 6377 (1956).
[28] Cf. Ref. 6, p. 78.
[29] D. M. S. Wheeler and J. W. Huffman, *Experientia* **16**, 516 (1960).

bands (237, 244, 252 mμ) of 22,25-oxidoholothurinogenin acetate, was demonstrated by an examination of the ORD curves obtained with VIIIb (17-keto) and its desoxymethylene derivative IXb. (Fig. 8.) The ORD curve of VIIIb exhibits a positive Cotton effect indicative[30,31] of a 13β, 14α configuration. The ORD curve of IXb is nearly the mirror image of VIIIb. The negative Cotton effect, observed with IXb corresponds to that reported[30,32] for other 17 keto-triterpenes with the 13α, 14α configuration. The inversion of configuration at C-13, after deformylation, is fully consistent with the observation that, where equilibration between C/D *cis* ↔ *trans*

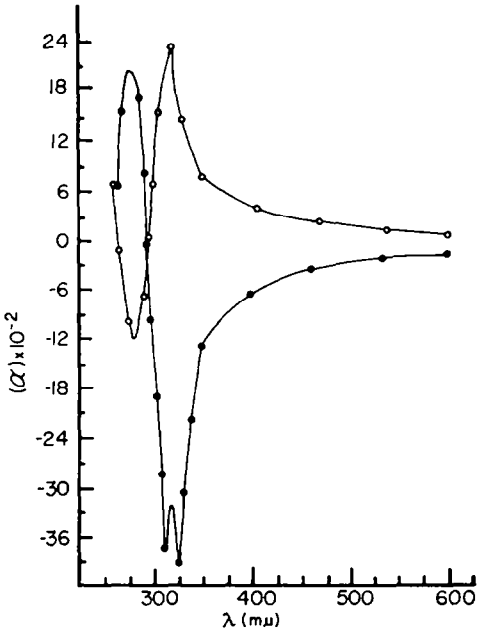


FIG. 8. ORD curves of VIIIb —o—o—o—, and IXb —●—●—●— in dioxan.

is possible, as in this instance, the preferred orientation is *cis*.[32] These assignments are fully confirmed by the NMR spectra of the compounds discussed below. 22,25-oxidoholothurinogenin therefore has its side chain and 17-hydroxyl group β and α oriented respectively.

C-20 *and* C-22 *Configuration.* The configuration at carbon atoms 20 and 22 are undetermined.

NMR spectra. The NMR spectra of the compounds derived from 22,25-oxidoholothurinogenin (Ia) and its 17-desoxy analog (IIa) are presented in Table 1. While the various assignments have been discussed or follow by analogy,[33] a few deserve special mention. For 22,25-oxidoholothurinogenin acetate (Ib), the assignment of the signals at 1·15 and 1·22 ppm to the methyl groups on C-10 and C-14 respectively is based on a comparison of its NMR with that of the 17-desoxy analog (IIb). The

[30] Ref. 24, p. 44, 58.
[31] J. Fishman and C. Djerassi, *Experientia* **16**, 138 (1960).
[32] J. Bielman, P. Crabbe and G. Ourisson, *Tetrahedron* **3**, 303 (1958).
[33] J. M. Lehn and A. Vystricil, *Tetrahedron* **19**, 733 (1963) and Refs cited therein.

comparable signals at 1·15 and 1·13 for compounds Ib and IIb respectively represent C-19, since this methyl group has the same environment in both compounds. The observed downfield shift, 0·22 ppm, for the methyl group at C-14 for 22,25-oxidoholothurinogenin (1·22) as compared to the 17-desoxy derivative (1·00) may be ascribed to the deshielding effect of the 17α-OH. A similar downfield displacement of a C-14 (α) methyl group arising from 1:3 diaxial interaction with a suitably disposed OH group (16α) on the D-ring has been reported.[34] The C-19 methyl signal (1·15 ppm) of 22,25-oxidoholothurinogenin is displaced downfield when compared to that of 24,25-dihydroagnosterol acetate (1·06 ppm). An examination of a Dreiding model of this compound reveals that the C-19 methyl falls within the deshielding-cone (plane)[33,35,36] of the carbonyl group of the lactone.

A comparison of the methyl signals of VIIIb 0·86 (6H), 0·95 (3H) and 1·05 (3H) ppm and its desoxy methylene derivative (IXb) 0·90 (6H), 0·98 (3H) and 1·32 (3H) ppm reveals a significant downfield displacement of the methyl group at C-14 (1·05 to 1·32 ppm). The downfield shift of this methyl group is a consequence of the change in configuration at C-13 ($\beta \rightarrow \alpha$). For IXb with C/D *cis* configuration the methyl at C-14 (α) falls into the deshielding cone[35] of the carbonyl group at C-17. Analogous downfield displacements of similarly placed methyl and carbonyl groups, resulting from *trans* to *cis* conversions have been reported.[33,37]

EXPERIMENTAL[38]

The UV in 95% EtOH and IR spectra were recorded with a Beckman D.U. and Perkin–Elmer IR 421 spectrophotometer respectively. NMR spectra were determined at 60 Mc/s with a Varian Associates spectrometer, A60. The chemical shifts δ are given in ppm relative to an internal trimethylsilane standard. M.ps were taken in an evacuated sealed tube (e.s.t.) or with a Fisher-Johns m.p. block (b.). Alumina IIN and alumina IIA refer to alumina prepared by the deactivation of Woehlm neutral alumina activity I with 3% by wt of water and 4% by wt of a 10% AcOH solution respectively. TLC on Woehlm neutral alumina, and benzene–AcOEt (9:1) as developing agent. For detection of spots, the plates were heated to 120°, sprayed first with a mixture of anisaldehyde–AcOH–H_2SO_4 (1:50:0·5); redried at 120°, sprayed with a mixture of AcOH–perchloric acid (1:1) and heated at 120° for 5 min.

Extraction and hydrolysis of holothurin

The following procedure for the extraction of holothurin from Cuvier's gland of *Actinopyga agassizi* is more convenient than that formerly employed[1]. Finely powdered Cuvier's gland (345 g, sun dried), dried by refluxing with benzene (3 l.) with a water separator, was dissolved in dry pyridine (2·7 l.) by gently warming the mixture. After standing over night (room temp) insoluble material was removed by centrifugation. The gummy glycoside mixture precipitated by the addition of Skelly B (5 l., b.p. 72–80°) was separated, refluxed with fresh Skelly B (1 hr) and the now solidified glycosides collected, yield 244 g. To a solution of the crude holothurin (150 g) in water (1·6 l.), maintained at 90°,

[34] D. Lavie, B. S. Benjaminov and Y. Shvo, *Tetrahedron* **20**, 2585 (1964). A striking example of a downfield shift of a methyl group caused by 1:3 diaxial interaction with an OH group is observed with fusidic acid. Cf. D. Arigoni, W. von Daehne, W. E. Godfredsen, A. Melera and S. Vangedahl, *Experientia* **20**, 344 (1964).

[35] L. M. Jackman, *Applications of NMR Spectroscopy in Organic Chemistry* pp. 122–124. Pergamon Press, London (1959).

[36] M. Amorosa, L. Caglioti, G. Guinelli, H. Immer, J. Keller, H. Wehrli, M. Mihailovic, K. Schaffner, D. Arigoni and O. Jeger, *Helv. Chem. Acta* **45**, 2674 (1962).

[37] C. W. Shoppee and R. E. Lack, *J. Chem. Soc.* 3611 (1964).

[38] Analyses by Dr. M. Manser, *Analytisches Laboratorium.* Basel, Switzerland and Schwarzkopf, Microanalytical Laboratory, Woodside, N.Y.

TABLE 1. NMR SPECTRA[a] (δ values in DCl_3)

Compound	Methyl groups at 4α, 4β	at 10β	at 14α	21	26, 27	$OCOCH_3$	3-H	7-H, 11-H	22-H	13-CH_2OAc
Ib	0·91, 0·99	1·15	1·22	1·38	1·26, 1·28	2·08	4·55	5·57, 5·25	4·23[b]	—
IIb	0·90, 0·96	1·13	1·00	1·35	1·22, 1·26	2·08	4·56	5·58, 5·29	4·08[b]	—
XVIIIb	0·90, 0·87	1·03	1·13	1·40	1·24, 1·27	2·08	4·48[c]	—	4·48[c]	—
XIX	1·08(6)	1·13 or 1·19	1·19 or 1·13	1·40	1·24, 1·27	—	—	—	4·25	—
VIb	0·88, 0·95	1·01	1·34(3) or 1·25(3)	1·38	1·25(6) or 1·25(3) and 1·34(3)	2·08(6)[e]	4·56	5·44(2)[c]	4·23	3·86(1)[d] and 4·30(1)[d]
VIIIb	0·90(6)	0·98	1·06	—	—	2·08(3) and 2·03(3)	4·56	5·50, 5·70	—	3·70(1)[d] and 4·33(1)[d]
IXb	0·86(6)	0·95	1·32	—	—	—	4·52	5·50, 5·90	—	—

[a] Signals attributable to 3-H, 7-H and 11-H are broad and centered as indicated, figures in ( ) give number of hydrogens. [b] Broad triplet. [c] Overlapping signals, centered as indicated and equivalent to two hydrogens. [d] Doublet with J = 12 c/s. [e] Inflection at 2·03.

a hot solution of HCl (670 ml. conc. HCl + 400 ml H_2O) was added slowly (0·5 hr) and the mixture stirred and heated (90–100°) for an additional 2–3 hr. The precipitated algycone mixture was separated by centrifugation. It retained large amounts of water and was extracted thoroughly with $CHCl_3$. Each extraction resulted in an emulsion which was broken by centrifugation. The combined extracts were washed with dil $NaHCO_3$aq, sat NaClaq, dried over Na_2SO_4, treated with Norite (10g) and Celite (30 g), and yielded, on evaporation to dryness, the crude aglycone mixture, holothurinogenins 43·5 g).

Preliminary separation of 22,25-*oxidoholothurinogenin* (Ia) *and* 17-*desoxy*-22,25-*oxidoholothurinogenin* (IIa) *from holothurinogenin* U (IIIa)

The aglycone mixture (20 g) was dissolved in boiling benzene (4 l.), filtered from insoluble material (not investigated) and the cooled solution chromatographed on a column of alumina IIA (300 g). The column was eluted successively with benzene, benzene–AcOEt mixtures and finally hot AcOEt. Fractions (500 ml) were collected and combined after an examination of their IR spectra, as summarized below.

Fraction	Eluent	Volume (l.)	Material (g)
I	Benzene	2·0	Oil
II	Benzene	5·0	Ia + IIa (5·70)
III	Benzene–AcOEt (2%)	3·0	Ia + ? (1·20)
IV	Benzene–AcOEt (10%)	2·0	IIIa + ?(2·95)
V	Benzene–AcOEt (10%)	5·5	IIIa(5·45)
VI	Benzene–AcOEt (25%)	1·0	IIIa + ?(0·35)
VII	AcOEt (hot)	1·0	?(1·35)

22,25-*Oxidoholothurinogenin acetate* (Ib) *and* 17-*desoxy*-22,25-*oxidoholothurinogenin acetate* (IIb)

Fraction II (Ia + IIa; 25 g) from several chromatographies (see above) was dissolved in a mixture of pyridine (260 ml) and Ac_2O (66 ml) and the mixture allowed to stand at room temp for 3 days. The solid acetate was precipitated by the addition of ice water, yield 27 g. The acetylated material (13·5 g) was dissolved in a mixture of benzene (150 ml) and Skelly B (150 ml) and chromatographed on alumina II N (300 g). Elution was effected with benzene–Skelly B mixtures and fractions (ca. 250 ml) were collected and combined according to their IR spectra.

Fraction	Eluent	Volume (l.)	Material (g)
I	Benzene–Skelly B (1:4)	0·75	IIb (0·45)
II	Benzene–Skelly B (1:4)	2·60	Ib + IIb (2·20)
III	Benzene–Skelly B (1:3)	3·50	Ib
IV	Benzene–Skelly B (1:1)	2·00	Ib (9·50)
V	Benzene	3·35	Ib

While each of the fractions, III–V, exhibited IR and UV spectra not significantly different from 22,25-oxidoholothurinogenin acetate (prepared below), the presence of isomeric material (side chain ?) and small amounts of a more unsaturated material(s) may be inferred from the following observations. 22,25-Oxidoholothurinogenin acetate, $[\alpha]_D$ +3°, was obtained in 15% yield after many crystallizations from MeOH; subsequent crops with essentially the same UV and IR spectra exhibited significantly lower rotations, $[\alpha]_D$ −20° to −75°, and showed a small maximum at 305 mμ (ε 500–1,000). The recrystallization of the combined fractions III–V (18·1 g from several batches) from MeOH gave the following results. Crop I: 8·7 g, $[\alpha]_D$ −11. Crop II: 2·7 g, $[\alpha]_D$ −17. Crop III: 4·6 g, $[\alpha]_D$ −57°. Crop IV: 1·3 g, $[\alpha]_D$ −86°. Crop V: 0·8 g, $[\alpha]_D$ −75°. Crop VI: 0·2 g, $[\alpha]_D$ −65°. Treatment of each of the above crops with AcOH–perchloric acid mixture and subsequent chromatography, as described below, gave pure 22,25-oxidoholothurinogenin acetate in essentially the same yield (80–90%). The method employed for the preparation of 22,25-oxidoholothurinogenin acetate follows.

Fractions III–V (1b; 12·35 g), combined from several experiments, dissolved in AcOH (600 ml)

containing perchloric acid (70%, 0·6 ml), was allowed to stand at room temp 2–3 days. The material precipitated on addition of ice water, was dissolved in benzene–Skelly B (3:1; 200 ml) and chromatographed on alumina II N. Elution with benzene–Skelly B mixture (3:1; 13 l.), gave after recrystallization from MeOH pure 22,25-oxidoholothurinogenin acetate (8·5 g); m.p. 289·2–289·6° (e.s.t.), $[\alpha]_D^{25}$ +5·5° ($CHCl_3$), λ_{max} 243 (ε 14, 950), λ_{sh} 237 (ε 13, 960), λ_{sh} 252 mμ (ε 10, 640), ν_{max} (CS_2) 3, 559 (OH), 3033, 814 (C=C*H*), 1777 (lactone), 1732, 1245, 1030, 975 (acetate), 1390, 1361 (gem-dimethyl), 1134 (side chain ether), 1087, 1074, 1058, 1030, 860 cm^{-1} (lactone + side chain ether). (Found: C, 72·88, 72·82; H, 8·60, 8·71. $C_{32}H_{46}O_6$ requires: C, 72·97; H, 8·80%.) TLC R_F = 0·30.

Repeated treatment of the purified acetate with AcOH–perchloric acid mixture, as above, did not effect any change in its properties. Some destruction of material was noted however. It was further observed that the acetate (Ib) has two allomorphic modifications. The acetate, as obtained from MeOH, on heating at 250–260° in an evacuated sealed tube for 1 hr undergoes a change, resulting in the new crystalline modification. The IR of the two forms in solution, CCl_4 or CS_2, were identical; in KBr significant differences were noted, as given below.

Ib	3525	3043	1773	1733	1186	1175	1150	1135	1067	1044 cm^{-1}
Ib "heated"	3555	3054	1784	1733	1198	1182	1165	1150	1077	1060 cm^{-1}

22,25-Oxidoholothurinogenin acetate (Ib), on heating in an evacuated sealed tube for a few hr 20° above its m.p. loses AcOH; which was identified by its IR. The acetate was recovered unchanged after treatment with pyridine–chromic oxide complex.

22,25-*Oxidoholothurinogenin* (Ia)

The pure acetate (Ib; 1 g) was hydrolyzed by refluxing in methanolic KOH (100 ml; 5%) for 5–6 hr and the product, precipitated by the addition of either water or dilute HCl. Recrystallization from AcOEt gave the aglycone (800 mg) m.p. 315·2–315·8° (e.s.t.), $[\alpha]_D^{25}$ −21·2° ($CHCl_3$), λ_{max} 244 mμ (ε 14,400), λsh 237 mμ (ε 13,340), λsh 252 mμ (ε 10,390), ν_{max} ($CHCl_3$) 3607, 3508 (OH), 812 (C=C—*H*), 1764 (lactone), 1131 (side-chain ether), 1080, 1058, 1035, 858 cm^{-1} (lactone + side-chain ether). (Found: C, 74·29; H, 9·15; mol. wt. 484 (mass spectroscopy). $C_{30}H_{44}O_6$ requires: C, 74·34; H, 9·15%; mol. wt. 484.) TLC R_F = 0·09.

Compound Ie (*bistrifluoroacetate*)

Compound Ia (350 mg) was added to a solution of dry pyridine (4 ml) and triflouroacetic anhydride (2 ml) prepared in the cold. The mixture was heated on a steambath under reflux for 3 hr and decomposed by the careful addition of ice water. The precipitate in benzene (15 ml) was passed rapidly through alumina II A (1 g) (protracted contact with alumina resulted in hydrolysis and recovery of the aglycone), and recrystallized from Skelly B, containing small amounts of AcOEt, m.p. 260–261° (b). λ_{max} 243 mμ (ε 15,300), no IR absorption in the hydroxyl region. (Found: C, 60·31; H, 5·95; F, 16·90. $C_{34}H_{42}O_7F_6$ requires: C, 60·34; H, 6·26; F, 16·85%.)

Compound Id (3,5-*dinitrobenzoate*)

A solution of Ia (200 mg) in dry pyridine (3 ml) containing 3,5-dinitrobenzoyl chloride (126 mg) was allowed to stand over night at room temp and subsequently heated at 85–90° for 1½ hr. The derivative obtained in the usual manner, after recrystallization from benzene melted with decomposition at 300·8–301·2° (e.s.t.). (Found: C, 65·32; H, 6·89; N, 4·33. $C_{37}H_{44}O_{10}N_2$ requires: C, 65·47; H, 6·83; N, 4·11%.)

Compound Ic (p-*toluenesulfonate*)

A mixture of Ia (1·0 g), *p*-toluenesulfonyl chloride (2·6 g), in dry pyridine (50 ml) was prepared in the cold and allowed to stand at room temp for 3 days. The crude product, precipitated with ice water, was recrystallized from AcOEt, yield 1·2 g. The m.p. of the recrystallized material was found to vary indiscriminately between 170–180° irrespective of the number of recrystallizations. (Found C, 69·53; H, 7·91; S, 5·19. $C_{37}H_{50}SO_7$ requires: C, 69·51; H, 7·90; S, 5·03.)

22,25-Oxidoholothurinogenone (IV)

The aglycone (Ia; 500 mg) was added, in the cold, to a solution of CrO_3 (500 mg) in pyridine (35 ml) and the mixture stirred 18 hr at room temp. Water was added and the product extracted with $CHCl_3$. The chloroform residue, in benzene, was chromatographed on alumina *IIA* (5 g), elution was effected with the same solvent (110 ml). The ketone (355 mg,) was recrystallized from AcOEt, m.p. 295–296°, $[\alpha]_D^{25}$ −41·6°, ($CHCl_3$), λ_{max} 244 mμ (ε 14,400), λ_{sh} 236 mμ (ε 13,430), λ_{sh} 252 mμ (ε 10,390), ν_{max} (CCl_4) 3567 (OH), 3030 (C=C—*H*), 1776 (lactone), 1709 (ketone), 1432 (—COC*H*$_2$), 1134 (side-chain ether), 1087, 1058, 1033, 853 cm^{-1} (lactone + side-chain ether). ORD in dioxan (*c*, 0·12–0·025), $(\alpha)_{600}$ −20°, $(\alpha)_{500}$ −44°, $(\alpha)_{333}$ −48°. $(\alpha)_{330}$ −32°, $(\alpha)_{322}$ +112°, $(\alpha)_{320}$ −220° (Fig. 7). (Found: C, 74·56, 74·65; H, 8·87, 8·85; mol. wt. 482 (mass spectroscopy). $C_{30}H_{42}O_5$ requires: C, 74·64; H, 8·77%; mol. wt. 482.)

Sodium boronhydride reduction of ketone (IV) *to aglycone* (Ia)

The ketone (IV; 200 mg) was dissolved in hot dioxan (10 ml) containing 1% by wt of water. The solution was cooled to room temp, $NaBH_4$ (60 mg) added, and the mixture was allowed to stand 2½ hr with intermittent swirling. Water (0·4 ml) was added and the reaction allowed to continue for an additional hr. The mixture was decomposed by careful addition of water and the precipitated aglycone (174 mg) proved to be identical in all respects, UV, IR, m.p., $[\alpha]_D$, with Ia. TLC on neutral alumina confirmed the homogeneity of the material.

Triene mixture (V) *by rearrangement of* Ic

A solution of the *p*-toluenesulfonate, Ic (2·2 g) and AcONa (2·12 g) in AcOH (200 ml) was heated on a steambath for 2½ hr. The mixture was taken to near dryness *in vacuo* and the product precipitated by the addition of water. The crude triene in Skelly B (400 ml) was passed through a short column of II A (2 g) and yielded on evaporation to dryness the triene mixture (1·2 g). Recrystallization from MeOH of a portion of this product yielded crystalline triene V (mixture), m.p. 215°, $[\alpha]_D^{25}$ −18·8°. λ_{max} 243·5 mμ (ε 14,300), λ_{sh} 239 mμ (ε 13,700), ν_{max} (CS_2) 3568 (OH), 3074, 3019, 813, 803 (C=C*H*$_2$ + C=C*H*), 1775 (lactone), 1131 (side-chain ether), 1086, 1056, 1031, 859 cm^{-1} (lactone + side-chain ether). The material was dried in high vacuum at 140°, but appeared to retain MeOH of crystallization. (Found: C, 76·36; H, 9·17; $C_{30}H_{42}O_4 \cdot \frac{1}{2}CH_3OH$ requires: C, 76·36; H, 9·15%.) The same triene mixture was obtained in very small yield by the action of PCl_5 on the aglycone (Ia). The principal products contained chlorine.

Acetone and formaldehyde from the triene mixture (V)

A mixture of the crude V (1·1 g) and OsO_4 (1·25 g) in dry pyridine (30 ml) was stirred in the dark for 10 days and evaporated to dryness under red. press. The osmate ester residue was decomposed by refluxing with an alcoholic KOH–mannitol mixture (benzene (18 ml) + MeOH (18 ml) + EtOH (18 ml), water (10 ml), mannitol (3·7 g), KOH (3·7 g)) for 3 hr. Na_2SO_3 was added and refluxing continued for an additional 3½ hr. The decomposition of the osmate ester, employing the milder conditions of Baran,[39] was not successful. The mixture was evaporated to dryness under red. press. and the residue extracted with both ether and $CHCl_3$. The combined extracts were washed with dil HCl, sat NaClaq, dried (Na_2SO_4), and gave on evaporation an amorphous product (1·17 g), which showed no significant absorption in the UV. The conjugated diene was also attacked by the OsO_4. A portion (534 mg) of this product and lead tetraacetate (1·77 g) in dry AcOH (25 ml) was stirred over night at room temp. Water (25 ml) was added, the mixture distilled and 15 ml of distillate was collected. Brady's reagent (75 ml) was added to the distillate and the precipitated 2,4-dinitrophenylhydrazone (48 mg), m.p. 152–163°, was collected after 2 hr. The filtrate was treated as described below. Recrystallization from EtOH gave pure formaldehyde 2,4-dinitrophenyl hydrazone, 27 mg, m.p. 164–166°, identical in all respects, IR, m.p., and mixed m.p., with an authentic sample.

The filtrate, after standing over night, was filtered from a small quantity of solid (acetate of

[39] J. S. Baran, *J. Org. Chem.* **25**, 257 (1960).

2,4-dinitrophenylhydrazine) and extracted thoroughly with pet. ether (b.p. 40–60°). The pet. ether extract was washed with water (3 × 50 ml), dried over Na_2SO_4 and evaporated to dryness. The residue in benzene (0·5 ml) was chromatographed on alumina II A (1 g) and eluted (as one band) with the same solvent. Recrystallization from EtOH gave acetone 2,4-dinitrophenylhydrazone, m.p. 126–127° (5 mg), identical in all respects, IR, m.p., mixed m.p. with an authentic sample.

The mixture, remaining after distillation (see above), showed in addition to absorption bands in the IR (CCl_4) for a lactone (1770 cm^{-1}), six-membered ring ketone (1713 cm^{-1}) and α,β-unsaturated ketone (1670 cm^{-1}) a strong band at 1743 cm^{-1}, indicative of a five membered ring ketone. Further purification of this material was unsuccessful.

ξ7,ξ8-Dihydroxy-22,25-oxidoholothurinogenin acetate (XVa)[40–42]

A mixture of Ib (420 mg, 0·80 m-mole) and OsO_4 (250 mg, 0·99 m-mole) in dry pyridine (6·5 ml) was stirred in the dark for 15 days. The mixture was decomposed[39] by stirring for ½ hr with a solution of $NaHSO_3$ (0·8 g) in H_2O (13 ml) and pyridine (8·7 ml) and extracted thoroughly with $CHCl_3$. The extract was washed with water, 0·1N HCl sat NaClaq and dried ($NaSO_4$). Its residue was crystallized from benzene to give the diol (300 mg), which lost benzene on prolonged drying at 144° in high vacuo; m.p. 278–280° (dec, b.), $[\alpha]_D^{25}$ −23·3, *end absorption* 209 mμ (ε 2,100), ν_{max} (KBr) 3553, 3524, 3464 (OH); 3032, 822, 786 () 1773 (infl.) and 1752 (lactone); 1733, 1248 (acetate). 1392, 1364 (gem-dimethyl), 1134 (side-chain ether), 1076, 1058, 1042, 867 cm^{-1} (lactone + side-chain ether). Found: C, 67·98; H, 8·58. $C_{32}H_{48}O_8$ requires: C, 68·54; H, 8·63%.)

Compound XVb (3, 7 *diacetate*)

The trihydroxy-compound (XVa; 88·4 mg) was allowed to stand in a mixture of dry pyridine (1 ml) and Ac_2O (0·4 ml) at room temp for 18 hr. The product, precipitated by the addition of water, was recrystallized from Skelly B–AcOEt mixture (10:1), m.p. 282–283° (rapid heating to 250°, b.), 272–274° (slow heating, b.), $[\alpha]_D^{25}$ +1·7°, *end absorption* 210 mμ (ε 1,550). ν_{max} (CS_2) 3575, 3548 (OH); 3033, 818 (*C*═*C*—*H*) 1774 (lactone), 1744–1729, 1241 1222, 1234 (infl.), 965 (diacetate), 1389, 1363 (*gem*-dimethyl), 1134, 1117 (side-chain ether), 1071, 1057, 1022 and 859 cm^{-1} (lactone + side-chain ether). The compound gave no coloration with tetranitromethane, atttributable[43] to the OH group α to the double bond. (Found: C, 67·62, 67·51; H, 8·47, 8·34. $C_{34}H_{50}O_9$ requires: C, 67·75; H, 8·36%.)

Compound XVII (11-*keto*-8:9-*ene*)

A subsequent repetition of the oxidation with a new batch of OsO_4 and recrystallization of the product from benzene gave only 20% yield of the 7:8 diol (XVa). Examination (IR, UV) of later crops indicated the presence of an α,β unsaturated ketone. Chromatography on alumina II A gave with benzene significant quantities of starting material and with benzene–AcOEt (8:2) the ketone (XVII) (20% yield). The ketone was recrystallized from MeOH, m.p. 331–331·6° (e.s.t.) $[\alpha]_D^{25}$ +61·8. λ_{max} 265 mμ (ε 7,500), ν_{max} ($CHCl_3$) 3560 (OH), 1766 (lactone), 1721 (acetyl), 1648, 1590 (α,β-unsaturated ketone), 1428, 1422 (α-methylene ketone), 1391, 1365 (*gem* dimethyl), 1134 (side-chain ether), 1072, 1058, 1042, 1024, 860 cm^{-1} (lactone + side-chain ether), ν_{max} (KBr) 1768 (lactone), 1729, 1243 (acetyl), 1638, 1585 (α,β-unsaturated ketone), 1409 cm^{-1} (α-methylene ketone). (Found: C, 70·31; H, 8·46. $C_{32}H_{46}O_7$ requires: C, 70·82; H, 8·54%.) Compound XVII did not form a 2,4-dinitrophenyl hydrazone on boiling with this reagent in methanolic HCl and the acetyl group was hydrolyzed (IR).

Compound XVI *by oxidation of* XVa

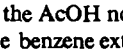
A mixture of XVa (250 mg) and lead tetraacetate (500 mg) in dry AcOH (12 ml) was stirred at room temp for 16 hr. The residue after removal of AcOH by lyophilization was decomposed with

[40] The positive value ΔM_D (XVb − XVa) = +129° observed on acetylation would indicate a 7β-OH configuration (cf. Ref. 41 and 42).

[41] J. Elks and G. H. Phillipps, *J. Chem. Soc.* 4320 (1956).

[42] L. F. Fieser and M. Fieser, *Steroids* pp. 239, 253. Reinhold, New York (1959).

[43] Ref. 42, p. 175.

water and the mixture again lyophilized. Neither the AcOH nor H_2O distillates gave a reaction with Brady's reagent. The product, obtained from the benzene extract of the residue, was recrystallized from Skelly C (b.p. 92–97°) to give the aldehyde (175 mg), m.p. 248–249° (b.), $[\alpha]_D^{25}$ +34·4°, λ_{max} 233 mμ (ε 7,500), ν_{max} (CCl_4) 3617, 3450 (OH), 3042 (), 2731, 2647 (weak) (aldehyde, *CHO*), 1772 (lactone), 1740–1729 (acetyl + aldehyde), 1681 (α,β-unsaturated ketone), 1390, 1365 (gem-dimethyl), 1131 (side-chain ether), 1099, 1080, 1058, 1050, 856 cm^{-1} (lactone + side-chain ether). (Found: C, 68·41; H, 8·29; $C_{32}H_{46}O_8$ requires: C, 68·79; H, 8·30%.)

Compound XIIIb (*7-keto-8:9-ene*)

(a) *By perbenzoic acid oxidation.* Compound Ib (200 mg, 0·38 mmole) in $CHCl_3$ (5 ml) containing perbenzoic acid (0·78 mmole) was allowed to stand in the cold (ice chest) 4 days. After 2 days the uptake of O_2 ceased and 1·26 equiv of O were absorbed. The $CHCl_3$ solution was washed with Na_2CO_3aq, dried over Na_2SO_4 and evaporated to dryness. The residue (160 mg) contained ca. 30% of the 7 keto-8:9-ene (XIIIb) as shown by its UV λ_{max} 254 mμ (ε 2,000), and IR. A solution of the residue in benzene (6 ml) containing BF_3–etherate (0·1 ml) was allowed to stand a few days at room temp. The deeply colored mixture was diluted with benzene, washed in the usual manner, and evaporated to dryness. The residue was reextracted with cold benzene and chromatographed on alumina II A (3 g). The ketone (40 mg) eluted with the same solvent (50 ml) was recrystallized from Skelly B and/or MeOH, m.p. 262–263° (e.s.t.) $(\alpha)_D^{25}$ +17° λ_{max} 256 mμ (ε 10,050), ν_{max} (CCl_4) 3568 (OH), 1770 (lactone), 1730, 1241, 976 (acetyl), 1662, 1595 (α,β-unsaturated ketone), 1422 (α-methylene ketone), 1391, 1365 (gem dimethyl), 1135, 1113 (side-chain ether), 1092, 1074, 1058, 1053, 1022, 860 cm^{-1} (lactone + side-chain ether); ($CHCl_3$) 3560 (OH), 1761 (lactone), 1722 (acetyl), 1652, 1591 (α,β-unsaturated ketone), 1416 (methylene ketone), 1391, 1365 (*gem*-dimethyl), 1134, 1113 (side-chain ether, 1090, 1073, 1056, 860 cm^{-1} (lactone + side-chain ether); (KBr) 1768 (lactone), 1735, 1245 (acetyl), 1660, 1594 (α,β-unsaturated ketone), 1419 (methylene ketone). (Found: C, 70·89, 70·65; H, 8·66, 8·82. $C_{32}H_{46}O_7$ requires: C, 70·82; H, 8·54%.)

The 2,4-dinitrophenylhydrazone was formed by refluxing (0·5 hr) a solution of the ketone with the reagent, in methanolic HCl (10 ml MeOH + 0·5 ml conc. HCl), m.p. 284–285°, λ_{max} 393 mμ (ε 20,800). The acetyl group was hydrolyzed in the formation of the derivative (IR).

(b) *By rearrangement.* Compound XVa (150 mg) in methanolic HCl (10 ml, conc. HCl 10% by vol) was allowed to stand at room temp 1–2 days. The mixture was evaporated under red. press. to a small volume and the product precipitated by the addition of water. Recrystallization from benzene gave the unacetylated XIIIa (3-hydroxy-7-keto-8:9-ene), m.p. 264–265°, $[\alpha]_D^{25}$ +15°. λ_{max} 256 (ε 8,300). IR ν_{max} ($CHCl_3$) 3612, 3560 (OH), 1763 (lactone), 1653, 1591 (α,β-unsaturated ketone). 1416 (methylene ketone), 1134, 1114 (side-chain ether). 1090, 1077, 1058, 860 cm^{-1} (lactone + side-chain ether) and no absorption for the acetyl carbonyl grouping. The crude product was acetylated by standing over night in a mixture of dry pyridine (3·5 ml) and Ac_2O (1 ml). The acetate, recovered by the addition of water, was dissolved in benzene (50 ml) and chromatographed on alumina II A (1·5 g). Recrystallization of the benzene eluate (200 ml) from MeOH gave XIIIb (64 mg), identical in all respects, IR, UV, m.p. with that obtained above. Further elution of the column with $CHCl_3$ yielded a small quantity of XVIII (11-keto-8:9-ene).

(c) *By oxidation of* Ib. To a stirred solution of Ib (520 mg) in AcOH (50 ml) a solution of CrO_3 (2·2 ml, 0·1 g/ml) in AcOH (90%) was added at room temp over a period of 2 hr. The mixture was heated at 40° (3 hr) and at 90° (1·5 min) and poured into ice water. The precipitate was dissolved in ether, the solution washed in the usual manner and evaporated to dryness. The residue in Skelly

Fraction	Eluent	Volume (ml)	Material
I	Skelly B–benzene (7:3)	500	Unconjugated 7-ketone
II	Skelly B–benzene (1:1)	600	Unconjugated 7-ketone + XIIIb
III	Benzene	200	Unconjugated 7-ketone + XIIIb + XII
IV	Benzene–AcOEt (2–10%)	650	XII

B–benzene (3:1; 8 ml) was chromatographed on alumina II A (10 g) and fractions (100 ml) collected and combined on the basis of their IR spectra.

The residues from fractions I–III (200 mg; the conjugated 7-keto-8:9-ene (1662 cm^{-1}) and the unconjugated 7-keto compound (1707 cm^{-1}) with the latter predominating) were combined, dissolved in Skelly B-benzene (7:3; 5 ml) and chromatographed on alumina II A (6 g). This mixture did not elute material as above; elution was effected with Skelly B-benzene (1:1; 1350 ml). Recrystallization from EtOH gave pure XIIIb (ca. 70 mg), identical in all respects with material prepared above. Isomerization presumably had taken place on the column, since little unconjugated ketone was recovered from the second chromatography.

ORD of XIIIb in dioxan (*c*, 0·19), $(\alpha)_{589}$ +20°, $(\alpha)_{378}$ +325°, $(\alpha)_{373}$ +310°, $(\alpha)_{360}$ +390°, $(\alpha)_{345}$ +185°, $(\alpha)_{301}$ −570°, $(\alpha)_{290}$ −465° (Fig. 7).

Compound XII (*7,11-diketo-8:9-ene*)

The residue (150 mg) of fraction IV was chromatographed on alumina II A (4 g). Elution with Skelly B-benzene (1:1; 100 ml) gave traces of XIIIb; Skelly B-benzene (1:1; 800 ml), benzene (350 ml) and $CHCl_3$ (20 ml) all gave the crude diketone. Recrystallizations from MeOH, containing a few drops of water, and finally pure MeOH yielded the yellow ene-dione (XII; 30 mg), m.p. 218–219°, λ_{max} 280 mμ (ε 6,390), ν_{max} (CCl_4) 3560 (OH), 1772 (lactone), 1734, 1241, 985 (acetate), 1680, 1684 (infl.) (conjugated carbonyls), 1423 (α-methylene ketone), 1392, 1365 (*gem* dimethyl), 1134, 1112 (side-chain ether), 1081, 1058, 860 cm^{-1} (lactone + side-chain ether). (Found: C, 69·57; H, 8·11. $C_{33}H_{44}O_8$ requires: C, 69·04; H, 7·97%.)

Compound XIV (*3-keto-7-keto-8:9-ene*)

A mixture of XIIIa (30 mg) and CrO_3 (30 mg) in dry pyridine (2 ml) was allowed to stand over night at room temp. The product, precipitated by the addition of water, was extracted with $CHCl_3$. The $CHCl_3$-solution was washed and dried in the usual manner. The residue, in benzene, was chromatographed on alumina II A. Elution with benzene afforded the ketone (XIV) m.p. 285–286°, λ_{max} 255 mμ (ε 9,100). No change was noted in its spectrum on the addition of alkali (alcoholic–KOH, 0·01N). ν_{max} (KBr) 3535 (OH), 1767 (lactone), 1704 (six-membered ring ketone), 1645, 1586 (ene-one), 1130, 1112 (side-chain ether), 1085, 1072, 1057, 1043, 855 cm^{-1} (lactone + side-chain ether); ($CHCl_3$) 3565 (OH), 1759 (lactone), 1702 (six-membered ring ketone), 1657, 1593 (ene-one), 1418 (α-methylene ketone), 1133, 1111 (side-chain ether), 1087, 1072 (infl.), 1056, 852 cm^{-1} (lactone + side-cahin ether).

Perhydro-22,25-oxidoholothurinogenin acetate (XVIIIb) and 3β-ethoxy-perhydro-22,25-*oxidoholothurinogenin* (XX)

Compound Ib (2·0 g) in glacial AcOH (200 ml) containing perchloric acid (70%; 0·2 ml) was hydrogenated in the presence of PtO_2 catalyst (600 mg) at atm press. After 24 hr additional catalyst (600 mg) was added and hydrogenation continued for 3 days. The mixture was filtered, fresh catalyst added (600 mg) and hydrogenation continued for an additional day. The absorption of H_2 was far in excess of that calculated for 3 moles. As previously reported,[28] under these conditions AcOH is hydrogenated. The hydrogenated products precipitated by the addition of water were redissolved in hot benzene (9 ml), Skelly B (110 ml) added, and chromatographed on alumina II A. Eluates (100 ml) were collected and combined according to their IR spectra.

Fraction	Eluent	Volume (ml)	Material (mg)
I	Skelly B	1000	(?) Oil (191)
II	Skelly B	500	XX (100)
III	Skelly B-benzene (3:1)	800	XVIIIb (600)
IV	Benzene; benzene–AcOEt (3:1)	1000	(?) (625)

Fraction III (600 mg) was recrystallized several times from MeOH to yield XVIIIb (330 mg), which gave no color with tetranitromethane, m.p. 267–268° (b.) $[\alpha]_D^{25}$ −9°, *end absorption* 210 mμ (ε 205), ν_{max} (CS_2) 3556 (OH), 1760 (lactone), 1730, 1241 (acetyl), 1387, 1363 (*gem*-dimethyl),

1133, 1118 (side-chain ether), 1082, 1069, 1054, 1047, 858 cm^{-1} (lactone + side-chain ether); (KBr) 3530 (OH), 1760 (lactone) 1729, 1245 (acetate), 1387, 1364 (*gem*-dimethyl), 1133 (side-chain ether), 1082, 1068, 1049, 859 cm^{-1} (lactone + side-chain ether). (Found: C, 72·40; H, 9·54. $C_{32}H_{50}O_6$ requires: C, 72·42; H, 9·54%.)

Fraction IV (625 mg) was rechromatographed on alumina II A (15 g), and elution with Skelly B-benzene (8:2; 1·6 l.) gave after recrystallization from MeOH an additional 100 mg of XVIIIb.

Fraction II (100 mg) was rechromatographed on alumina II A (20 g) and eluted in the following manner.

Fraction	Eluent	Volume (ml)	Material
A	Petrol ether (b.p. 40–60°)	1100	(?) Oil
B	Skelly B-benzene (9:1)	1000	Like Fraction I (IR) (see above)
C	Skelly B–benzene (9:1)	200	XX

Several recrystallizations of Fraction C from petrol ether (30°–60° b.p.) gave the pure XX, which gave no coloration with tetranitromethane, m.p. 253–254° (b.), $[\alpha]_D^{25}$ +3·4°, *end absorption* 209 mμ (ε 290), IR ν_{max} (CS_2) 3556 (OH), 1761 (lactone), 1131 (side-chain ether), 1104 (—OC_2H_5), 1083, 1067, 1047, 869 (lactone + side-chain ether). No absorption for an acetyl group was observed. (Found: C, 74·40; H, 10·29. $C_{32}H_{52}O_5$ requires: C, 74·38; H, 10·14%.)

Perhydro-22,25-*oxidoholothurinogenin* (XVIIa)

Compound XVIIIb (350 mg) was hydrolyzed by refluxing in alcoholic KOH (50 ml; 2½%) as described for the unsaturated acetate (Ib). Recrystallization from MeOH gave the aglycone (XVIIIa), m.p. 321–322 (e.s.t.), after drying at 130° in high vacuo, $[\alpha]_D^{25}$ −19·9°, ν_{max} ($CHCl_3$) 3611, 3555 (OH), 1751 (lactone), 1133, 1112 (side-chain ether). (Found: C, 73·53; H, 9·74. $C_{30}H_{48}O_5$ requires: 73·73; H, 9·90%.)

Perhydro-22,25-*oxidoholothurinogenone* (XIX)

The perhydro derivative XVII a (300 mg) was oxidized with CrO_3 (300 mg)–pyridine (22 ml) mixture as described for Ia. The crude keto product, in benzene, was chromatographed on alumina II A (4 g) and eluted with benzene (200 ml). Recrystallization from AcOEt afforded XIX (230 mg), m.p. 310–310·5° (e.s.t.), $[\alpha]_D^{25}$ −34·2°, ν_{max} ($CHCl_3$) 3560 (OH), 1751 (lactone), 1695 (3-ketone), 1133, 1111 (side-chain ether), 1069, 1058, 1045, 857 cm^{-1} (lactone and side-chain ether); (KBr) 3530 (OH), 1760 (lactone), 1700 (3-ketone). ORD in dioxan (*c*, 0·05), $(\alpha)_{650}$ −20°, $(\alpha)_{589}$ −24°, $(\alpha)_{500}$ −32°, $(\alpha)_{400}$ −60°, $(\alpha)_{350}$ −104°, $(\alpha)_{316}$ −236°, $(\alpha)_{290}$ −52° (Fig. 7). (Found: C, 74·20; H, 9·25. $C_{30}H_{46}O_5$ requires: C, 74·04; H, 9·53%.)

Perhydro-22,25-*oxidoholothurinogenin* (XVIIIa) *by reduction of ketone* XIX

Perhydro-22,25-oxidoholothurinogenone (100 mg) dissolved in dioxan–H_2O (10 ml, 1%) was reduced with $NaBH_4$ (50 mg), as described for IV. Recrystallization from MeOH afforded the alcohol (66 mg), identical in all respects, mixed m.p., specific rotation, IR with perhydro-22,25-oxidoholothurinogenin as obtained above.

Compound VIb (LAH *reduction of* Ib)

A solution of Ib (3·18 g) in dry tetrahydrofurane (150 ml) was added over a period of 45 min to a boiling stirred suspension of LAH (4 g) in tetrahydrofurane (300 ml). The mixture was refluxed for 2 hr, additional LAH (3 g) and tetrahydrofurane (125 ml) added, and the reaction continued for 2½ hr. After standing over night at room temp, the mixture was decomposed, in the cold, by the cautious addition of AcOEt (15 ml), followed by sat Na_2SO_4aq (20 ml). Solid Na_2SO_4 (20 g) was added, the mixture centrifuged and the supernatant collected. The inorganic precipitate was extracted with tetrahydrofurane (2 × 100 ml) and finally ether (3 × 200 ml). The combined organic extracts were dried over Na_2SO_4, evaporated under red. press., the residue, crude VIa, exhibited a triple maxima in the UV at 236, 244 and 252 mμ and showed no carbonyl absorption in the IR. The product,

after standing over night at room temp in a mixture of pyridine (35 ml) and Ac_2O (10 ml), was heated for 1 hr on a steambath. The acetylated product precipitated by addition of ice water was collected, dissolved in benzene, the benzene solution washed in the usual manner and evaporated to dryness. The residue, in Skelly B (20 ml), was chromatographed on alumina II A (20 g), and elution was effected with Skelly B (400 ml); Skelly B–benzene (95:5; 300 ml); Skelly B–benzene (1:1; 400 ml). The Skelly B eluate gave on recrystallization from pet. ether VIb (1·8 g), m.p. 212–213 (b.) $[\alpha]_D^{25}$ +54°, triple maxima λ_{max} 244 (ε 17,250), 236 (ε 14,900), and 252 mμ (ε 11,370). ν_{max} (CS_2) 3572, 3448, (OH), 3030, 810 (>C=CH–), 1735, 1752 (infl.), 1710 (infl.), 1241, 1235 (acetate), 1133, 1104 cm^{-1} (side-chain ether). (NMR see Table I). (Found: C, 71·11, 71·36; H, 9·21, 9·08. $C_{34}H_{52}O_7$ requires: C, 71·30; H, 9·15.)

The Skelly B–benzene eluate (95:5) furnished an additional 50 mg of VIb. The Skelly B–benzene (1:1) eluate (0·6 g), amorphous material, not affected by lead tetraacetate, was not further investigated.

Compound Xa *from* VIb *or* VIa

Compound VIb (140 mg) was hydrolyzed by refluxing for 3 hr in methanolic KOH (10 ml); 2½%). The mixture was evaporated to a small volume under red. press. and the crude VIa (100 mg) precipitated by the addition of ice water. Triple maxima λ_{max} 236 (ε 11,250), 244 (ε 16,500), 252 mμ (ε 11,200), ν_{max} (CCl_4) 3620, 3400 (OH); (KBr) 3050, 3015, 807 cm^{-1} (C=CH); no carbonyl absorption. To a vigorously stirred and cooled solution of VIa in MeOH (20 ml)–water (5 ml) mixture cold conc. HCl (7·5 ml) was added slowly, and the reaction allowed to proceed for an additional 2 hr at room temp. The rearranged Xa (81 mg), which precipitated during the course of the reaction, was recrystallized from MeOH. m.p. 264–265° (b.), $[\alpha]_D^{25}$ +61. The product showed strong *end absorption* in the UV. 210 mμ (ε 3,940), ν_{max} (CCl_4) 3620, 3400, 3365 (OH), 1129, 1102, 1084, 862 cm^{-1} (side-chain +18, 11 ethers). The product still retained one mole of MeOH after drying at 80° *in "vacuo"*. (Found: C, 71·49; H, 9·82; OCH_3, 6·15. $C_{30}H_{48}O_6 \cdot CH_3OH$ requires: C, 71·50; H, 10·05; OCH_3, 5·96%.) Drying at higher temps removed the MeOH only in part.

A solution of VIb (50 mg) in methanolic HCl (10%; 5 ml) was stirred over night at room temp. The precipitated product (16·6 mg) was identical in all respects with Xa obtained above; hydrolysis and rearrangement had taken place. Compound VIa was recovered from the mother liquor.

Compound Xb

A solution of Xa (40 mg) in pyridine (3 ml) and Ac_2O (1 ml) was allowed to stand at room temp 2 days. The crude acetate precipitated by addition of water was recrystallized from Skelly B; m.p. 248–249°, $[\alpha]_D^{25}$ +70·2°; ν_{max} (CS_2) 1733, 1245 (acetyl), 814 cm^{-1} (>C=CH–). (Found: C, 72·46; H, 9·60. $C_{32}H_{50}O_6$ requires: C, 72·42; H, 9·50%.)

Compound XIa *from* Xa (This experiment was performed by Dr. R. Ledeen)

A mixture of Xa (300 mg) and lead tetraacetate (342 mg), in glacial AcOH, (20 ml), was stirred at room temp for ca. 14 hr and the mixture taken to dryness by lyophylization. The residue was decomposed by the addition of dil. KIaq, the slight I_2 coloration discharged with $Na_2S_2O_3$, steam distilled, and extracted with boiling Skelly B (3 × 20 ml). The Skelly B extract was taken to dryness and its residue extracted with warm CS_2. The residue of the CS_2 extract was redissolved in boiling CCl_4 (20 ml), the solution treated with norite, and evaporated to dryness. The product (60 mg) was recrystallized several times from acetone, containing a small amount of water and gave a positive tetranitromethane test; m.p. 200–201°, $[\alpha]_D^{25}$ +139, λ_{max} 290 (ε, 36), *end-absorption* 208 (ε 3,700); ν_{max} (CS_2) 3620 (OH), 1736 (five-membered ring ketone); (KBr) 3476 (OH), 1720, 1409 cm^{-1} (five membered α-methylene ketone), 3037, 812 (>C=C(H)–), 1154, 1097 cm^{-1} (18, 11 ether). (Found: C, 76·65; H, 9·21. $C_{32}H_{52}O_3$ requires: C, 76·70; H, 9·36%.)

Compound XIb

A solution of XIa (60 mg) in Ac_2O (0·5 ml) and pyridine (1 ml) was allowed to stand at room temp for 2 days. After working up in the usual manner, the product in Skelly B (5 ml) was

chromatographed on alumina II A (1 g) and elution effected with the same solvent (35 ml). Several recrystallizations from pet. ether, 40–60°, furnished pure material; m.p. 181–182° (b.), change of crystal form at 126°, $[\alpha]_D^{25}$ +145° ($CHCl_3$), *end absorption* 210 mμ (ε 3,720); ν_{max} (CS_2) 1735 (acetyl + five-membered ring ketone), 1390, 1361 (*gem*-dimethyl); 1154, 1090(18, 11 ether), 811 cm^{-1} ($\diagdown C{=}C{-}H \diagup$); (KBr) 1734, 1725 cm^{-1} (acetyl + five-membered ring ketone). Found: C, 74·68; H, 8·72. $C_{24}H_{48}O_4$ requires: C, 74·58; H, 8·87%.)

Compound VIIIb (*C_{22}-ketone*)

A mixture of VIb (500 mg), lead tetraacetate (1·0 g), in dry AcOH (25 ml) was stirred at room temp for 24 hr. The solution was lyophilized and the AcOH distillate (A) collected (see below). The residue was decomposed by the addition of cold water, the mixture lyophilized, and the water distillate (B) collected (see below). The residue was extracted with pet. ether (5 × 20 ml). The crystalline residue from the combined extract was redissolved in Skelly B (5 ml), and chromatographed on alumina II A (8 g). The product, VIIIb (200 mg), was eluted with Skelly B (200 ml), Skelly B–benzene (8:2; 100 ml) and recrystallized from pet. ether and/or MeOH; m.p. 163–164°, $(\alpha)_D^{25}$ +107° ($CHCl_3$) λ_{max} 242 (ε 16,600) λ_{sh} 236 (ε 15,100) λ_{sh} 250 mμ (ε 10,000); λ_{max} (CS_2) 3030, 813 (2- $\diagdown C{=}CH_2 \diagup$) 1745, 1736 (infl.) (acetyl + five-membered ring ketone), 1409 (α-methylene ketone) 1390, 1363 cm^{-1} (*gem*-dimethyl); no band for hydroxyl group. ORD in dioxan (*c*, 0·20), $(\alpha)_{589}$ +90°. $(\alpha)_{330}$ +820°, $(\alpha)_{318}$ +2370°, $(\alpha)_{298}$ +730°, $(\alpha)_{280}$ −1215°, $(\alpha)_{270}$ +290° (Fig. 8). (Found: C, 72·64; H, 8·56; mol. wt. 428 (mass spectroscopy). $C_{26}O_{36}O_5$ requires: C, 72·87; H, 8·47%; mol. wt. 428.)

Compound VIIa, *osazone of* VII (*C_8-ketone*)

Brady's reagent (50 ml) was added to distillate A, after 24 hr the crude osazone (33 mg), m.p. 241°, was collected. To the filtrate, distillate B and Brady's reagent (500 ml) were added and the next day the second precipitate of osazone (33·5 mg), m.p. 235°, removed. Subsequent additions of Brady's reagent (4 × ca. 50 ml) gave over a period of a week a mixture (418 mg) of the osazone and the acetate of 2,4-dinitrophenylhydrazine. The mixture was refluxed with EtOH (200 ml), and the insoluble osazone (56 mg), m.p. 240°, combined with the first two crops, yield 120 mg (25% of theoretical). Recrystallization from $CHCl_3$ gave analytical material, m.p. 243–244°, identical in all respects, IR, UV, mixed m.p., with an authentic sample prepared below. λ_{max} ($CHCl_3$), 395 mμ (ε43, 700), λ (infl.) 420–450 mμ (ε 36,400). (Found: C, 46·18, 45·86; H, 4·50, 4·29; N, 20·84, 21·24; O, 27·26. $C_{30}H_{32}N_8O_8 \cdot H_2O$ requires: C, 46·15; H, 4·65; N, 21·53; O, 27·60%.)

The EtOH extract, see above, on evaporation to a small volume yielded the acetate of 2,4-DNPH, m.p. 196–198°, identical in all respects, m.p., IR, with an authentic sample prepared by acetylation of 2,4-DNP.

Compounds VIIb *and* VIIc

To each distillate A and B, obtained from a second oxidation of VIb (200 mg), Brady's reagent (75 ml) was added and the mixtures extracted, after 5–8 min, with pet. ether (200 ml). The combined extracts recrystallized from MeOH gave a crystalline solid (19 mg), m.p. 118–119° (soft at 75°). The residue of the mother liquor yielded, from CS_2, a few crystals of m.p. 154–155°, (not investigated) and an oil (O), (see below).

The material, of m.p. 118–119°, was chromatographed on alumina II A (2 g). On development with Skelly B a distinct separation of the material into two yellow bands on the column was observed. The faster moving band was eluted with Skelly B and the slower one with Skelly B-benzene (9:1). The Skelly B eluate recrystallized from MeOH gave material (1·2 mg) of m.p. 114–115°, which did not give the osazone (VIIa) on heating with excess Brady's reagent. The Skelly B–benzene eluate, recrystallized from MeOH, gave VIIc (2·1 mg), m.p. 129–130°, IR (CS_2) 3,330 (NH) and 3220 cm^{-1}, (bonded OH and NH (broad)). Compound VIIc in EtOH (1·5 ml) and Brady's reagent (2 ml) were refluxed for 1–2 hr, the precipitated osazone in $CHCl_3$–benzene (1:1), was passed through alumina

II A. (500 mg). Recrystallization from $CHCl_3$ gave the osazone (VIIa,) identical in all respects, m.p., IR, UV with an authentic sample, prepared below.

The oily derivative "O" (see above) was chromatographed on alumina II A. On development with Skelly B and Skelly B–benzene mixtures, four distinct bands were observed on the column. Bands I and II, eluted with Skelly B–benzene (9:1), yielded no osazone on heating with Brady's reagent. Band III, eluted with Skelly B–benzene (8:2), gave a small amount of VIIc. Band IV, eluted with benzene–Skelly B (1:1), the main component, was an oil which exhibited in the IR, (CS_2), absorption at 3325 cm^{-1} (NH), no bands attributable to OH or bonded NH, gave the osazone VII on heating with Brady's reagent, and is formulated as the hydrazone (VIIb).

Synthesis of VIIa (*osazone of 6-methylhept-5-ene-2,3-dione*)

Ethyl nitrite generated from $NaNO_2$ (25 g) was bubbled over a period of ½ hr through a solution of 6-methylhept-5-ene-2-one (25 g), (b.p. 85–86°/39 mm; n_D^{20} 1·4352) in CCl_4 (25 ml), previously saturated with dry HCl. The reaction mixture was maintained at 45–55° throughout the addition. The mixture was cooled, poured into excess $NaHCO_3$aq and extracted with ether (200 ml). The extract was re-extracted with 10% NaOHaq (6 × 50 ml) and the combined alkaline extracts carefully acidified at 5° with conc HCl. The precipitated nitroso compound was isolated with ether and the ether extract after treatment in the usual manner gave on evaporation to dryness the crude nitroso derivative (2 g). A portion of the crude nitroso compound (220 mg), in EtOH (125 ml) and Brady's reagent (250 ml), was heated on the steam bath for 1 hr. The precipitated osazone, in $CHCl_3$ (150 ml), was passed through alumina II A (8 g). Recrystallization from $CHCl_3$ gave VIIa (300 mg), m.p. 243–244° (b.) λ_{max} 395 mμ (ε 43,800), λ (infl.) 420–450 (ε 36,400); ν_{max} (KBr) 2970 (broad), 2930 (broad), 2868, 1074, 1054, 958, 937, 921; (Nujol) 3590, 3450 cm^{-1} (both weak and broad; water of crystallization). (Found: C, 45·58; H, 4·72; N, 21·20. $C_{20}H_{22}N_8O_8 \cdot H_2O$ requires: C, 46·15: H, 4·65; N, 21·53%.)

Compound XXIV (*osazone of 6-methylheptan-2,3-dione*)

Commercial 6-methylheptan-2-one (25 g) (found by refractive index to contain ca. 30% of the unsaturated 5-ene) in EtOH (95%; 125 ml) was hydrogenated (30 lb, room temp) in the presence of Pd–C catalyst (5%; 3 g) until absorption of H_2 had ceased. Distillation gave the pure ketone (20 g), b.p. 164°, n_D^{20} 1·4104. The DNP derivative, prepared in the usual manner and recrystallized from pet. ether, melted at 83·5–84·5°, as reported. The pure heptanone (25·2 g) was reacted with $EtNO_2$, as described above, and gave in good yield the nitroso ketone (14·2 g). The osazone was prepared and recrystallized from $CHCl_3$, as described, for the unsaturated analog. In addition to the absence of any bands in the IR attributable to water of crystallization small but significant differences were noted in the spectra of the saturated and unsaturated osazones, both with respect to wavelength and intensity; ν_{max} (KBr) 2945, 2921, 1061, 1046, 962, 938, 921 cm^{-1}. (Found: C, 74·74; H, 4·74; N, 22·30; O, 25·14. $C_{20}H_{24}N_8O_8$ requires: C, 47·61; H, 4·79; N, 22·20; O, 25·37%.)

Compound VIIIa (*by hydrolysis of* VIIIb *at room temp*)

Compound VIIIb (108 mg) was initially dissolved in hot MeOH (3 ml), the solution cooled, methanolic conc. HCl (20%; 3 ml) added, and the mixture allowed to stand at room temp for 2 days. The product (63 mg) precipitated by the addition of water, was recrystallized from dioxan–water mixture containing a small amount of HCl, m.p. 197–198°, λ_{max} 242 mμ (ε 16,040), $\lambda_{(infl)}$ 236 (ε 14,480), $\lambda_{(infl)}$ 252 (ε 9,845); ν_{max} (CCl_4) 3631, 3460 (broad) (OH), 1735 (five-membered ring ketone), 1409 cm^{-1} (α-methylene ketone); (KBr) 3040, 811, 789 cm^{-1} (). (Found: 75·37; H, 9·35. $C_{22}H_{32}O_3 \cdot \frac{1}{2}H_2O$ requires: C, 75·43; H, 9·39%.) On reacetylation with pyridine–Ac_2O mixture the starting VIIIb was obtained.

Compound IXb *and formaldehyde from* VIIIb

(a) A solution of VIIIb (100 mg) in a mixture of MeOH (9 ml) and conc HCl (1 ml) was refluxed for 4 hr with N_2 passing through the solution. The effluent gas was bubbled through Brady's reagent and the precipitated 2,4-dinitrophenylhydrazone (20 mg; 50% yield), m.p. 166°, proved identical in all respects, mixed m.p., IR, with that of formaldehyde. (Under the same conditions a solution

containing 3·7 mg of formaldehyde gave in the effluent gas a 50% recovery of formaldehyde, based on the DNPH derivative (12 mg) recovered.) To the methanol HCl solution water was added and the precipitated unacetylated IXa collected (60 mg); m.p. ca. 171°, λ_{max} 240 mμ (broad) (ε 11,200) ν_{max} (CCl_4) 3607 (OH), 3033 (>C=C<H), 1745 (five-membered ring ketone), 1402 cm^{-1} (α-methylene ketone). The crude IXa in dry pyridine (1 ml) and Ac_2O (0·1 ml) was allowed to stand at room temp under N_2 2 days. The mixture was evaporated under red. press. to near dryness, water added and the acetylated product extracted with pet. ether (b.p. 30–60°). Chromatography on alumina II A (1 g) and elution with pet ether (100 ml) gave after recrystallization from the same solvent IXb (30 mg), m.p. 164° (marked m.p. depression on admixture with VIIIb). λ_{max} 236 mμ (ε 10,900); ν_{max} (CS_2) 3040, 3027, 818, 796 (>C=C<H), 1744 (five-membered ring ketone), 1402 (α-methylene ketone) 1733, 1240, 1028 cm^{-1} (acetyl). No band for (OH). ORD in dioxan (*c* 0·112–0·022), $(\alpha)_{600}$ −208°. $(\alpha)_{589}$ −214°, $(\alpha)_{321}$ −4050°, $(\alpha)_{314}$ −3180°, $(\alpha)_{309}$ −3740°, $(\alpha)_{276}$ +2040°, $(\alpha)_{265}$ +680°, Fig. 8). (Found: C, 76·92; H, 9·06. $C_{22}H_{32}O_2$ requires: C, 77·49; H, 9·05%.)

(b) *Quantitative determination of formaldehyde*[44] *from* VIIIb. A mixture of VIIIb (0·0917 mg), suspended in chromotropic reagent was heated, with frequent shaking, in a sealed tube on a steam bath for 2½ hr. The intensity of color developed was measured with a Klett photoelectric colorimeter employing a 540 mμ filter. A standard concentration curve was made with formaldehyde under the same conditions. (Found: 0·16 μM formaldehyde, theoretical = 0·21 μM (75% recovery.)

Equilibrium studies

(a) *compounds* XIa ⟷ VIIIa. Separate equimolar solutions (6·70 × 10^{-5} M) of XIa and VIIIa in methanolic HCl (10%) were incubated at 37° and the changes in their optical densities, log (I_0/I), were noted at three wavelengths over a period of 5 days. Equilibrium between the two compounds was demonstrated as summarized below.

Compound	mμ	Log I_0/I (Days) (0)	(4)	(5)
XIa	230	0·005	0·664	0·680
	242	0·000	0·878	0·892
	250	0·000	0·667	0·667
VIIIa	230	0·750	0·697	0·688
	242	1·072	0·885	0·859
	250	0·712	0·620	0·610

(b) *Compounds* Xa ⟷ VIa. A solution of the rearranged product Xa (8·82 × 10^{-5} M) in methanolic HCl (10%) was incubated at 37° and the change in density at various wavelengths was recorded with time, as summarized below.

Compound	mμ	Log I_0/I (Days) (0)	(3)	(5)	
Xa	230	0·005	0·945	0·955	(0·900)[a]
	242	0·000	1·385	1·404	(1·380)[a]
	250	0·000	1·000	0·980	(0·914)[a]

[a] Calculated Log I_0/I for VIa.

[44] W. R. Frisell, L. A. Meech and C. G. Mackenzie, *J. Biol. Chem.* **207**, 709 (1954).

Compound VIIIb (C_{32}-*ketone*) *from holothurinogenin* U (IIIa) (Fig. 6)

Holothurinogenin U (IIIa; 10 g; fraction V of crude holothurin chromatographies) was acetylated and chromatographed on alumina II A (40 g) as described for Ia. Recrystallization, of the benzene eluate, from AcOEt gave an acetate mixture (8 g) m.p. 262–271°; λ_{max} 244, λ_{sh} 237 and 252 mμ; ν_{max} (CH_2Cl_2) 3600, 3490 (OH), 1762, 1720 cm^{-1} (lactone plus acetyl); (TLC and analysis C, 74·25; H, 9·06 suggest a mixture of $C_{32}O_5$ and $C_{33}O_6$ derivatives). The mixture was reduced with LAH reacetylated and chromatographed on alumina II A as described for IIb. Recrystallization from Skelly B, of the Skelly B–benzene (9:1) eluate, gave reduction product (2·2 g), which could not be purified by further recrystallizations, albeit the m.p. was raised to 161–168°. The reduction product (1·1 g) was oxidized with lead tetraacetate (2·2 g) and the volatile and non-volatile ketone separated as described. The non-volatile ketone mixture was chromatographed on alumina II A and furnished after recrystallization from Skelly B compound VIIIb (375 mg), identical in all respects with the sample prepared from 22,25-oxidoholothurinogenin acetate.

Compound XXIV (Fig. 6)

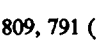
The mother liquor of the first crystallization of VIIIb (above) gave on evaporative distillation a few mg of liquid ν_{max} (CCl_4) 1745, 1741, 1719 (acetoxy ketone $CH_3{\cdot}CO{\cdot}CH(OCOCH_3)(CH_2)_3CH(CH_3)_2$?). This material in EtOH (30 ml) and Brady's reagent (30 ml) was heated on a steambath for 1½ hr. The precipitated osazone, XXIV, m.p. 243° was identical in all respects, IR mixed m.p. with the authentic sample of the saturated osazone (XXIV) prepared from 6-methylheptan-2,3-dione.

Compounds XXII, XXIII and XXIV (*volatile ketone mixture*)

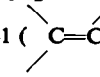
Brady's reagent (75 ml) was added to the AcOH distillate and after 1 hr the precipitated hydrazones were dissolved in benzene and chromatographed on alumina II A (3 g). Six separate bands were clearly developed on the column however, only the fastest moving component, eluted with Skelly B–benzene (7:3; 150 ml), gave sufficient material for identification. This eluate after rechromatography gave impure 6-methylheptanone DNP derivative (XXII) m.p. 76°, rep. 83–84°, mixed m.p. 76–77°. Positive identification was made by the correspondence of their IR spectra.

The water distillate, after reaction with Brady's reagent for ½ hr was extracted thoroughly with pet. ether. The organic extract was chromatographed on alumina II A (2 g), and elution effected with Skelly B–benzene (7:3). Eluate I (400 ml) gave the impure DNP derivative of XXIII. Eluate II (200 ml) gave a hydrazone which exhibited absorption in the IR at 1745 cm^{-1}, indicative of an acetyl group and yielded on refluxing with Brady's reagent the saturated osazone (XXIV), m.p. 241°, identical in all respects with an authentic sample.

17-*Desoxy*-22,25-*oxidoholothurinogenin acetate* (IIb)

Fractions I and II (6·2 g from several chromatographies of holothurinogenin acetate mixture) was treated with an AcOH–perchloric acid mixture, as described for 22,25-oxidoholothurinogenin acetate. The recovered acetate, dissolved in a mixture of benzene (35 ml), Skelly B (105 ml) was chromatographed on alumina II N (120 g) and elution effected with Skelly B–benzene (3:1; 6 l.), and Skelly B–benzene (1:1, 5 l.). Recrystallization from MeOH gave pure material (3·5 g); m.p. 266·2–266·5° (e.s.t.), $[\alpha]_D^{25}$ +21·3°. λ_{max} 244 (ε 14,900), λ_{sh} 237 (ε 13,700), λ_{sh} 255 mμ (ε 10,570), ν_{max} (CS_2) 3040, 809, 791 () 1768 (lactone), 1733, 1241, 973 (acetyl); 1390, 1361 (gem-dimethyl), 1131, 1117 (side-chain ether); 1058, 1014, 858 cm^{-1} (lactone + side-chain ether); no band for (OH) group. (Found: C, 75·22; H, 8·82. $C_{32}H_{46}O_5$ requires: C, 75·26; H, 9·08%.) TLC R_F = 0·55.

17-*Desoxy*-22,25-*oxidoholothurinogenin* (IIa)

The acetate IIb (500 mg) was hydrolyzed and worked up as described for Ib. Recrystallization from MeOH gave pure material, m.p. 285·8–286·4° (e.s.t.), $[\alpha]_D^{25}$ −9·3°, λ_{max} 244 (ε 16,800), λ_{sh} 237 (ε 15,300), λ_{sh} 252 (ε 11,800); ν_{max} (CCl_4) 3632 (OH), 3041 (), 1771 (lactone), 1133, 1118

(side-chain ether), 1052, 852 cm^{-1} (lactone + side-chain ether). (Found: C, 76·55; H, 9·32. $C_{30}H_{44}O_4$ requires: C, 76·88; H, 9·46%.) TLC $RF = 0·14$.

17-*Desoxy*-22,25-*oxidoholothurinogenone* (XXI)

Compound IIa (130 mg) was oxidized with CrO_3 (170 mg), pyridine (15 ml) mixture and the ketone isolated as described for Ia. Recrystallization from AcOEt afforded pure material (67 mg), m.p. 282–283° (b.). $[\alpha]_D^{25}$ −24·8°. λ_{max} 243 mμ (ε 14,000), λ_{sh} 237 mμ (ε 12,600), λ_{sh} 252 mμ (ε 10,000); ν_{max} (CCl_4) 3030 () 1770 (lactone), 1709 (six-membered ring ketone), 1134, 1118 (side-chain ether). 1059, 1020, 860 cm^{-1} (side-chain ether + lactone). (Found: C, 77·06; H, 8·98; O, 14·10. $C_{30}H_{42}O_4$ requires: C, 77·21; H, 9·07; O, 13·71%.)

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