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STRUCTURE OF GENTIOPICROSIDE*

L. CANONICA, F. PELIZZONI, P. MANITTO and G. JOMMI
Istituto di Chimica Industriale della Università di Milano, Italia

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Abstract—A new formula for the gentiopicroside (I), the principal bitter glucoside of common gentians, is proposed.

THE gentiopicroside (gentiopicrin of the old nomenclature), which is the principal bitter glucoside of common gentians,† was isolated in 1862[1] and has since been the object of several chemical studies. In connection with this problem, the work of several investigators[2–11] can be summarized as follows. The gentiopicroside ($C_{16}H_{20}O_9$; I), formed from the aglycone protogentiogenin ($C_{10}H_{10}O_4$; II) and β-glucose as indicated by its hydrolysis with emulsin, is not stable to acids and alkali and rather unstable even in neutral solution. On acetylation, it gives the tetra-acetylgentiopicroside (III), from which the glucoside (I) is regenerated but in poor yield. The extraction of the gentiopicroside must be carried out on fresh roots. It is very difficult and gives better results if carried out on the acetylderivative (III).

The instability of the gentiopicroside made the study of its structure extremely difficult. The primary genin (II) has never been isolated. The chemical hydrolyses were unsuccessful while with emulsin three derivatives of II are obtained in insignificant yields. The first product, obtained under controlled conditions (mesogentiogenin), is an optically inactive oil with the same U.V. absorption spectrum as the glucoside (I). After more prolonged enzymatic hydrolysis, two products are obtained: the dimeric gentiogenin $(C_{10}H_{10}O_4)_2$ and the eugentiogenin which, as shown by its spectroscopic properties, is derived by rearrangement of the original molecule (II).

These aglycones were not useful in the study of the structure of gentiopicroside. The gentiopicroside has three double bonds, (λ_{max} 270 mμ; log ε 3·96), two of which are rapidly hydrogenated to give the tetrahydrogentiopicroside (IV) (λ_{max} 247 mμ; log ε 3·89). The third double bond is hydrogenated more slowly, and at the same time the molecule is hydrolysed into β-glucose and the fully hydrogenated aglycone, the hexahydroprotogentiogenin ($C_{10}H_{16}O_4$; V) with no absorption in the U.V. The

* Preliminary Communication: *Tetrahedron Letters* No 24, 7 (1960).

† It has been found in: *G. lutea* A. Kromeyer, *Arch. Pharmaz* **110**, 27 (1862); *G. asclepiadea* M. Bridel, *C. R. Acad. Sci., Paris*, **155**, 1164 (1912); *G. punctata* M. Bridel, *Ibid.*, **156**, 627 (1913); *G. cruciata* M. Bridel, *Pharm. et Chim.* [7], **7**, 392 (1913); *G.pneumonthae* H. Bourquelot and M. Bridel, *Ibid.* [7], **2**, 149 (1910); *Chlora perfoliata* H. Bourquelot and M. Bridel, *C. R. Acad. Sci., Paris*, **150**, 114 (1910); *G. scabra* Y. Asahina and Y. Sakurai, *Ber. Dtsch. Chem. Ges.* **69**, 771 (1936); *Swertia perennis* M. Bridel, *J. Pharm. Chim.* [7], **6** 481 (1912).

[1] A. Kromeyer, *Arch. Pharmaz*, **110**, 27 (1862).
[2] G. Tanret, *C. R. Acad. Sci., Paris* **141**, 207 (1905).
[3] G. Tanret, *Bull. Soc. Chim.* [3], **33**, 1059 (1905).
[4] Y. Asahina, J. Asano, Y. Tanase and Y. Ueno, *Chem. Ber.* **69**, 771 (1936).
[5] Y. Asahina and Y. Sakurai, *Chem. Ber.* **72**, 1534 (1939).
[6] Y. Sakurai and K. Yoshina, *J. Pharm. Soc. Japan* **71**, 55 (1951); *Chem. Abstr.* **46**, 2499 (1952).
[7] F. Korte, *Chem. Ber.* **87**, 512 (1954).
[8] F. Korte, *Chem. Ber.* **87**, 769 (1954).
[9] F. Korte, *Chem. Ber.* **87**, 780 (1954).
[10] Private communication, Y. Sakurai, *Chem. Ber.* **87**, 780, (1954).
[11] L. Canonica and F. Pelizzoni, *Gazz. Chim. Ital.* **87**, 1251 (1957).

tetrahydroglucoside (IV) gives with emulsin the aglycone ($C_{10}H_{14}O_4$) tetrahydroprotogentiogenin (VI). The tetrahydroaglycone (VI) has a conjugated double bond (λ_{max} 247 mμ; log ε 3·96), gives a *p*-nitrophenylhydrazone, has an active hydrogen atom, gives a red colour with diazonium salts and, according to Korte, gives a positive reaction with ferric chloride but according to Asahina *et al.* this last reaction is negative. On catalytic hydrogenation, VI gives the hexahydrogenine (V).

The catalytic hydrogenation of the acetylgentiopicroside (III) gives a mixture of two isomers: the α-tetra-acetyltetrahydrogentiopicroside (VII) and the β-tetra-acetyltetrahydrogentiopicroside (VIII). The relationship between them is discussed later. The two acetyltetrahydroderivatives VII and VIII give on catalytic hydrogenation the same tetra-acetylhexahydrogentiopicroside (IX). This tetra-acetylhexahydrogentiopicroside (IX) gives, on acid hydrolysis, the same aglycone (V) which is formed by the catalytic hydrogenation of the aglycone (VI) and gentiopicroside.

It has been established that the glucoside (I) and all its derivatives contain a lactone group, but because this is very stable, it led to the hypothesis, later shown to be uncorrect, that it is a γ-lactone function.

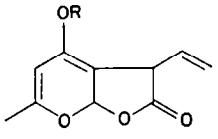
One of the two remaining oxygen atoms of the aglycone moiety of gentiopicroside, must be assigned to the glucosidic linkage. On account of the apparent enol behaviour of the tetrahydroaglycone (VI), this hydroxyl group was supposed to be of enolic type. The remaining oxygen atom, has been ascribed by all the A.A. to a cyclic ether, as its behaviour precludes the possibility of a hydroxy, alkoxy or carbonyl group. The degradation reactions carried out on gentiopicroside and its derivatives give only compounds with few carbon atoms. The fact that these are obtained only in very low yields made their identification uncertain.

Two degradation reactions, however, proved significant in the elucidation of the structure of I, namely the pyrolysis of the tetrahydroglucoside (IV) in good yield (70%) to n-butyric aldehyde and the ozonolysis of the gentiopicroside to formaldehyde. The fact that formaldehyde is obtained on ozonolysis of I but not of its hydrogenated derivatives shows that the gentiopicroside contains a terminal methylene group. The two tetrahydroisomers VII and VIII give, on boiling with 1 N sulphuric acid, carbon dioxide and acetone. This reaction which has a complex and obscure mechanism, led us to an incorrect formulation. None of the formulae proposed by Sakurai (X), Korte (XI) and ourselves (XII_a and XII_b) in previous work, is compatible with all these experimental facts.

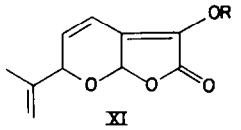
The structure X does not account for the U.V. absorption spectra of I or its derivatives. The structure XI does not explain the formation of the two tetrahydroacetylglucosides VII and VIII, which cannot be considered as stereoisomers owing to the fact that they have different U.V. spectra (λ_{max} 247 and 232 mμ respectively). On the other hand, the hypothesis[7] of a molecular rearrangement with formation of a new hydroxy group, is not acceptable because the same hexahydroglucoside (IX) is obtained by hydrogenation of both VII and VIII. Moreover VIII is not acetylated under usual conditions and its I.R. spectrum does not show hydroxyl bands. It follows that the difference between the two compounds VII and VIII can only be ascribed to the chromophore systems, derived from the conjugation of the one lactone carbonyl to two different double bonds. As a consequence, it must be admitted that the carbon atom in α-position to the carbonyl is ramified.

A further argument against structure XI is the fact that synthetic compounds of

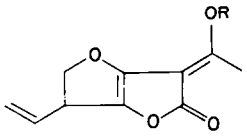
the type XIII containing the skeleton of the hexahydroaglycone derived from XI, have chemical and spectroscopical properties different from those of the hexahydroprotogentiogenin.[12–15]



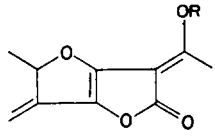
X



XI



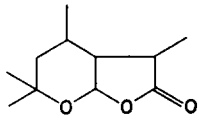
XII a



XII b

R = β-D-glucose

The structures XII_a and XII_b also contain a γ-lactone, but the I.R. spectra show the presence of a saturated δ-lactone in the hexahydroaglycon V (1746 cm^{-1} in Nujol; 1730 cm^{-1} in KBr) and in the hexahydroglucoside IX (1740 cm^{-1} in Nujol); and a α-β unsaturated δ-lactone in the acetylgentiopicroside III and in the two isomeric tetrahydroderivatives VII and VIII (1725, 1704, 1706 cm^{-1} in $CHCl_3$). This discrepancy between the proposed formulae and the spectroscopic evidence has already been



XIII

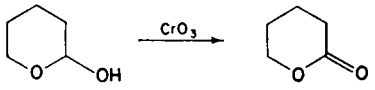
indicated by Korte[16]. Besides, the formulae XII_a and XII_b are neither compatible with the behaviour of hexahydroprotogentiogenin, nor, as it will be seen later, with the structure of some of its derivatives.

As a result of the present work, a new formula for the gentiopicroside, which accounts for all the experimental evidence so far obtained, is proposed. The hexahydroprotogentiogenin (V; $C_{10}H_{16}O_4$) obtained in good yield on acid hydrolysis of the glucoside (IX), under controlled conditions, has m.p. 134°, $[\alpha]_D$ +153° ($CHCl_3$). The literature[4] gives m.p. 140° and $[\alpha]_D$ +161° (alcohol). It does not show absorption maxima in the U.V. above 210 mμ and its I.R. spectrum shows bands at 3330 cm^{-1} (associated hydroxyl), at 1746 cm^{-1} (saturated δ-lactone). On titration, it behaves as a monovalent lactone; it reduces silver nitrate in ammonia solution but does not reduce

[12] L. Canonica, E. Fedeli and A. Castelnuovo, *Gazz. Chim. Ital.* **87**, 998 (1957).
[13] F. Korte, K. H. Büchel and L. Schiffer, *Chem. Ber.* **91**, 759 (1958).
[14] F. Korte and H. Machleidt, *Chem. Ber.* **90**, 2276 (1957).
[15] F. Korte and K. Trautner, *Fortschritte der Chemie Organischer Naturstoffe* Vol. 17, p. 179. Wien (1959). G. Büchi arrived at the same structure. Private communication (23 January 1961).
[16] *Fortschritte der Chemie Organischer Naturstoffe* Vol. 17, p. 132. Wien (1959).

triphenyltetrazolium chloride in the cold and with *p*-nitrophenylhydrazine and 2,4-dinitrophenylhydrazine does not form any definite derivative. The C-alkyl determination according to Kuhn–Roth[17] corresponds to one C-alkyl, propionic acid separating by V.P.C. On cromic acid oxidation in acetone,[18] it gives a di-δ-lactone (XIV; $C_{10}H_{14}O_4$), m.p. 93°, $[\alpha]_D$ −24° ($CHCl_3$), which has no absorption in the U.V. and contains active hydrogen atoms[4] or carbonyl groups. It has no reducing properties and on titration uses two equivalents of alkali. The I.R. spectrum shows no hydroxyl bands and one strong band at 1730 cm^{-1} (saturated δ-lactone).

The dilactone (XIV) differs from the tetrahydroaglycone (VI). Of the two δ-lactone groups in XIV, one is present in the hexahydroaglycone (V) and the other is derived on oxidation of a 2-hydroxytetrahydropirane.



The presence of a 2-hydroxytetrahydropirane ring in the molecule of V is confirmed by formation of a methylether (XV)[9] on reaction with methanol in the presence of hydrochloric acid.

The methylether (XV), m.p. 113°, $[\alpha]_D$ +208° has no absorption in the U.V. and its I.R. spectrum shows no hydroxyl band but it has a band at 1730 cm^{-1} (saturated δ-lactone). Compound XV reduces neither Tollens reagent nor triphenyltetrazolium chloride in the cold nor does it react with carbonyl reagents but it titrates as a monovalent lactone. It can also be obtained from the hexahydroglucoside (IX) by methanolysis and it reforms the aglycone (V) by action of dilute mineral acids. The hexahydroprotogentiogenin (V) on prolonged heating *in vacuo* yields a dehydrated compound (XVI; $C_{10}H_{14}O_3$), m.p. 69°, $[\alpha]_D$ +126° ($CHCl_3$) which has no absorption maxima in the U.V. (in cyclohexane). The I.R. spectrum shows no hydroxyl band but has a band at 1660 cm^{-1} (enol double bond) and at 1725 $^{-}cm^{1}$ (unconjugated δ-lactone). Compound XVI, on treatment with methanol in the presence of hydrochloric acid, gives the methylether (XV).

The properties of V, XIV, XV and XVI show that the hexahydroaglycone (V) contains the following ring systems, which must share two carbon atoms:

In the gentiopicroside and its glucoside derivatives, the sugar is attached to the hemiacetyl hydroxyl of the aglycone moiety. Considerable rotational differences exist between the glucosides IX and IV and their aglycones V (or its methylether XV) and VI respectively. The comparison cannot be extended to compounds I and II owing to the impossibility of isolating the aglycone II.

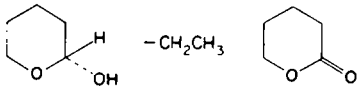
$$[M]_D \text{ (IV)} - [M]_D \text{ (VI)} : -696 \text{ (in alcohol)}$$
$$[M]_D \text{ (IX)} - [M]_D \text{ (V)} : -640 \text{ (in } CHCl_3\text{)}$$
$$[M]_D \text{ (IX)} - [M]_D \text{ (XV)} : -779 \text{ (in } CHCl_3\text{)}$$

[17] C. F. Garbers, H. Schmid and P. Karrer, *Helv. Chim. Acta* 37, 1336 (1954).
[18] K. Bowden, I. M. Heilbron, E. R. H. Jones and B. C. L. Weedon, *J. Chem. Soc.* 39 (1946).

These rotational differences can be explained by admitting that the removal of the glucose is accompanied by an inversion of the configuration of the hemiacetyl carbon atom. The magnitude of this effect agrees with the values observed when passing from a β-2-hydroxytetrahydropirane to an α-2-hydroxytetrahydropirane.[19] This supports

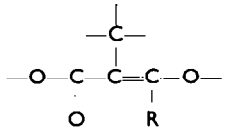
(β) (α)

the assignment of a β-2-hydroxytetrahydropirane configuration to the hemiacetyl carbon atom, which is the only asymmetric centre of the aglycone moiety of the gentiopicroside. The two remaining carbon atoms of the hexahydroaglycon (V) belong to an ethyl side-chain as shown by the formation of propionic acid in the C-alkyl determination. The ethyl group is formed during the reduction of the vinyl side-chain of I. In hexahydroprotogentiogenin the following groups are present:

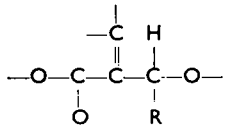


The relative positions of the functional groups must now be determined. Useful information may be obtained from the spectroscopic properties of the tetrahydroacetylglucosides VII and VIII. From what is known of the spectra of α–β unsaturated lactones[20] and esters,[21] the α-isomer (λ_{max} 247 mμ; log ε 4·02) should be a β-alkoxy-α,β-unsaturated lactone. The hypothesis that it could be an α–alkoxy–α, β-unsaturated lactone is excluded by the presence of a ramification in the α-position to the carbonyl. The oxygen atom of the β-alkoxy-group cannot be the hemiacetyl hydroxyl of the glucosidic linkage. In fact the elimination of the hemiacetyl hydroxyl from the aglycone (V) results in the formation of a double bond which as shown by U.V. and I.R. evidence is not conjugated to the lactone system. Therefore the oxygen atom of the β-alkoxy group can only be that of the pirane ring.

The α-acetyltetrahydrogentiopicroside (VII) contains the group:



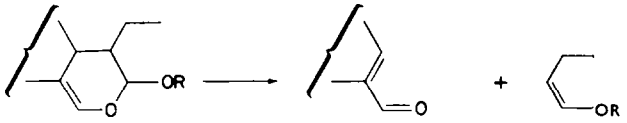
and the β-isomer (VIII):



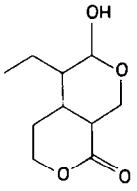
The mild hydrolysis of the tetrahydroaglycone (VI) yields formic acid, and this is a good indication that R is a hydrogen atom. The pyrolysis of the tetrahydroglucoside

[19] O. Halpern and H. Schmid, *Helv. Chim. Acta* **41**, 1109 (1958).
[20] F. Korte, J. Falbe and A. Zschocke, *Tetrahedron* **6**, 201 (1959).
[21] F. E. Bader, *Helv. Chim. Acta* **36**, 215 (1953).

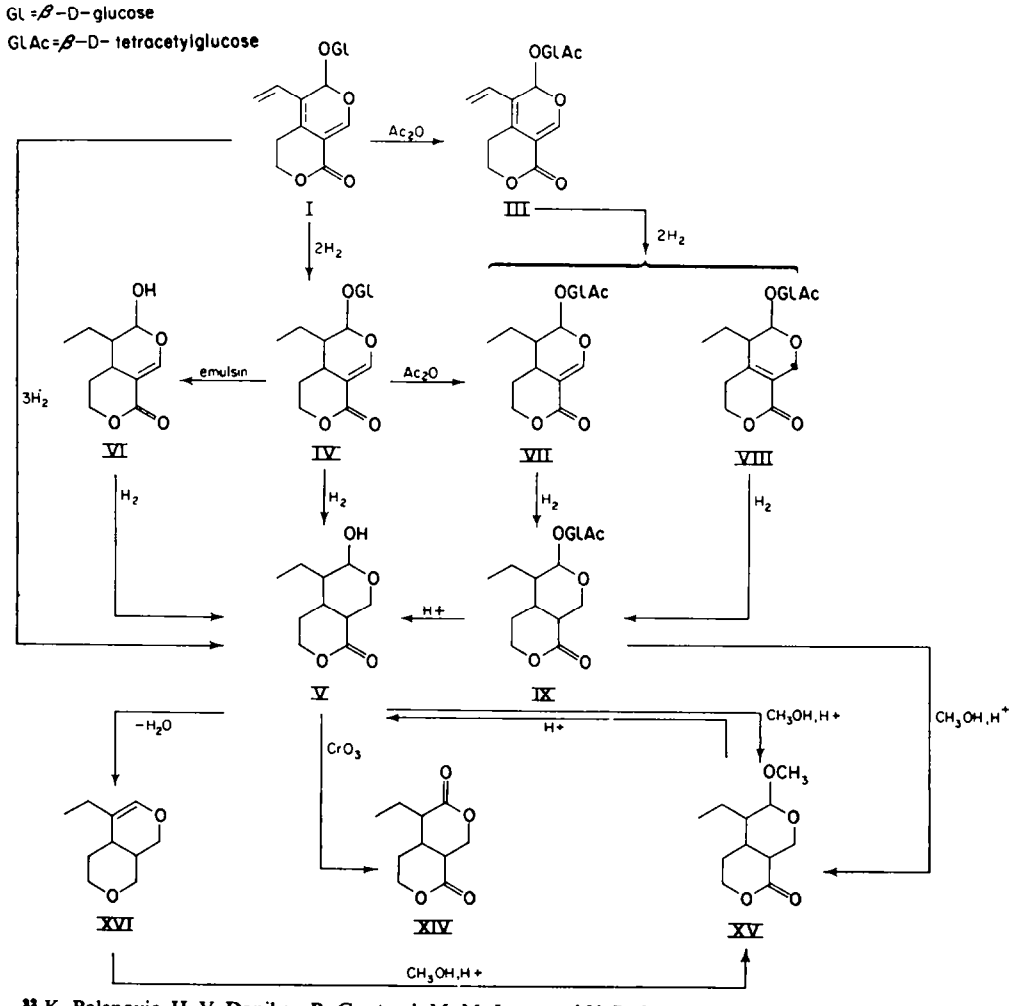
(IV) gives butyric aldehyde, a reaction similar to that obtained with the dihydrobakankosine[22] and, therefore, interpreted as a retro Diels Alder and establishes the



position of the side chain. Therefore, the final structure of V is

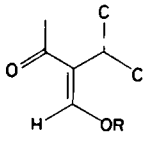


and the structures for gentiopicroside (I) and the related products are:



[22] K. Balenovic, H. V. Daniker, R. Goutarel, M. M. Janot and V. Prelog, *Helv. Chim. Acta* **35**, 2519 (1952).

These structures are further substantiated by the NMR spectra. The NMR evidence confirms the presence of an ethyl side-chain in XV, VII and VIII (centred triplet at 54·5 and 61 cycles/sec from the TMS reference, for the CH_3^- of XV and VII, and of VIII respectively). The ethyl group is not present in the acetylgentiopicroside (III). The electronic integration shows that the methylether (XV) contains five protons on carbon atoms linked to an etheral oxygen, while the dilactone (XIV) contains only four such protons. The α-tetrahydroacetylglucoside (VII) contains one olefinic proton (centred doublet at 452 cycles/sec with J ~2 cycles/sec) which can be attributed to a system such as the following:



This system is present also in the acetylgentiopicroside (III). The NMR spectrum of the β-isomer (VIII) shows the absence of olefinic protons and its electronic integration shows twelve protons on carbon atoms linked to one (or two) etheral oxygens. The position of the ethyl side-chain is confirmed by the NMR spectrum of the dehydrated product (XVI), the CH_2 group signal is displaced ~30 cycles/sec to low field in comparison with the compounds in which the ethyl side-chain is not adjacent to a double bond.[*]

EXPERIMENTAL

Hydrogenation of III *to* IX. The tetra-acetylgentiopicroside (III) (2·5 g), dissolved in 250 ml of methanol or ethanol, was hydrogenated, for 4½ hr, in the presence of 2 g of 10% Pd–C, at room temp and 40 atms. The catalyst was filtered off and the solvent evaporated to dryness under reduced press. The residue was treated with diethylether (30 ml) and filtered. The crude product (IX) thus obtained (1·95 g; 77%) had m.p. 155° and showed no absorption in the U.V. above 210 mμ. After crystallization from benzene–ligroin and chromatography on neutral alumina (activity III; elution with benzene), the m.p. of the product was 161–161·5°. $[\alpha]_D$ −63° (*c*,1% in $CHCl_3$). The chromatography had to be carried out very rapidly to avoid considerable loss.

Hydrogenation of VII *to* IX. Compound VII (0·5 g) (λ_{max} 247 mμ; log ε 4·02) was dissolved in 200 ml of ethanol and hydrogenated for 4 hr in the presence of 10% Pd–C (0·5 g) at room temp and 30 atms. The catalyst was filtered off and the solvent evaporated under reduced press. The residue was treated with ethylether (6 ml) and 0·382 g of crude (IX) were obtained (76%), m.p. 154–155°, showing no absorption maxima in the U.V. above 210 mμ. After crystallization from benzene–ligroin the product had a m.p. 161–161·5° $[\alpha]_D$ −63° (*c*, 1% in $CHCl_3$).

Hydrogenation of VIII *to* IX. Compound VIII (0·5 g) (λ_{max} 232 mμ; log ε 3·82) was dissolved in 200 ml of ethanol and hydrogenated, for 4 hr in the presence of 10% Pd–C (0·5 g) at room temp and 30 atms. The catalyst was filtered off and the solvent evaporated under reduced press. The residue was treated with ethylether (6 ml) and 0·470 g (93·6%) of crude IX were obtained, m.p. 154–155°, showing no absorption maxima in the U.V. above 210 mμ. After crystallization from benzene–ligroin the product had a m.p. 161–161·5°. $[\alpha]_D$ −63° (*c*, 1% in $CHCl_3$). The infra-red spectrum is identical to that of IX obtained by hydrogenation of VII. A mixed m.p. of the two products gave no depression.

Hydrolysis of IX *to* V. Compound IX (1·943 g) was suspended in a mixture of dioxane, water and

* We are greatly indebted to Dr. A. Melera for the measurement of the NMR spectra. Measurements were carried out on a Varian 4302, 60 megacycles spectrophotomer with electronic integrator, in the Research Laboratory of the Varian A. G., Zürich. Samples were dissolved in $CDCl_3$ and TMS was used as internal reference.

sulphuric acid (35:65:2 in vol). The mixture was heated to boiling, under vigorous stirring, and in a few minutes a clear solution was obtained. This was refluxed for 20 min and then cooled in ice. After saturation with sodium chloride, the aqueous solution was extracted with chloroform or methylene chloride (10 times for 30 ml). The organic extracts were dried on sodium sulphate and the solvent evaporated under reduced press. The residue was treated with ethylether (5 ml) and filtered. Crude V (0·519 g; 70%), m.p. 132°, was obtained. $[\alpha]_D$ +150° (*c*, 1% in $CHCl_3$). After crystallization from benzene–petroleum ether, the m.p. was 134° and the $[\alpha]_D$ +153°. The literature gives m.p. 140° and $[\alpha]_D$ +161° (in alcohol). (Found: C, 60·09; H, 7·96; C–C_2H_5, 19·4; Calcd. for $C_{10}H_{16}O_4$: C, 60·00; H, 8.00; C–C_2H_5, 20·5%).

Titration: Compound V (49·7 mg; 0·2485 mmole) was dissolved in 5 ml 0·1 N NaOH and left at room temp for 24 hr. The excess of NaOH was titrated with 0·1 N H_2SO_4 2·54 ml being used. Compound V reduces silver nitrate in ammonia solution but does not reduce triphenyltetrazolium-chloride in the cold nor does it show absorption maxima in the U.V. above 210 mμ.

Cromic acid oxidation of V *to* XIV. Compound V (1 g) was dissolved in 100 ml acetone. The solution was cooled at 0° and treated, dropwise (30 min with a good stirring) with 10 ml cromic acid mixture (10·3 g CrO_3, 8·7 ml H_2SO_4 and 30 ml water). Stirring was continued for 10 min at room temp and the excess of oxidant destroyed by addition of 50 ml methanol. The solution was diluted with water (500 ml) and extracted 10 times with 100 ml methylene chloride. The extracts were dried with sodium sulphate and evaporated to dryness under reduced press. The residue (0·697 g; 70·4%) m.p. 90°, crystallized from di-isopropylether yielding 0·414 g of pure XIV m.p. 92–93°. From the mother liquor a further amount of less pure compound (0·197 g, m.p. 90–93°) separated. (Found: C, 60·56; H, 7·26. calc. for $C_{10}H_{14}O_4$: C, 60·59; H, 7·12%). U.V.: no absorption above 210 mμ; $[\alpha]_D$ −23·6 (*c*, 1% in $CHCl_3$; literature gives −30·53 in ethanol). *Titration:* 197·3 g (0·996 mmole) was titrated with 0·1 N NaOH (required 9·92 ml). An excess of 20 ml of 0·1 N NaOH was added and the solution after standing 12 hr was again titrated with 0·1 N H_2SO_4, 9·90 ml being required. (The volume of NaOH used for the second equivalent is 10·1 ml).

Dehydration of V *to* XVI. Compound V (1 g) was distilled at 0·5 mm on a bath kept at 140–150°. The distillation was continued for 4 hr and the product (0·625 g; 68%) solidified, on cooling. The crystallization from ethylether yielded 0·335 g of XVI, m.p. 69° and from the mother liquor an additional 0·260 g, m.p. 63–67° was obtained. (Found: C, 65·71; H, 7·57. $[\alpha]_D$ +126 (*c*, 1% in $CHCl_3$); calc. for $C_{10}H_{14}O_3$: C, 65·91; H, 7·74%). *Titration:* 183 mg in 20 ml 0·1 N NaOH were titrated after 4 hr, 9·65 ml of 0·1 N H_2SO_4 being used.

Methanolysis of IX *to* XV. Compound IX (3 g) in 200 ml 0·1 N HCl and methanol was dissolved. The solution was allowed to stand at room temp for 24 hr and excess silver carbonate added. The mixture was filtered and the solvent evaporated to dryness under reduced press. The residue was treated with water and benzene and the two layers separated. The aqueous phase was extracted twice with benzene and the benzene extracts dried and evaporated. An oily residue (1·123 g) was dissolved in benzene and chromatographed on neutral alumina (activity III). Elution with benzene gave first an oily fraction (0·146 g) and then a crystalline product, m.p. 90–105° (0·551 g; 45·5%). On continuing the elution with benzene–ethylether (3:1), a further amount of the crystalline product mixed with an unidentifiable oily substance (0·237 g) was obtained. The chromatography had to be carried out very rapidly to avoid considerable loss of material. The crude product could be crystallized from a small volume of methanol or from benzene–petroleum ether. $[\alpha]_D$ + 208° (*c*, 1% in $CHCl_3$).

(Found: C, 61·82; H, 8·52; O–CH_3, 14·34; calc. for $C_{11}H_{18}O_4$: C, 6168; H, 8·41; OCH_3, 14·48%). The pure compound XV (m.p. 113°) has no absorption maxima in the U.V. It does not reduce silver nitrate in ammonia solution nor triphenyltetrazolium chloride and was titrated as described for the other compounds by adding an excess of aqueous NaOH and titrating the excess alkali after 12 hr at room temp.

Hydrolysis of XV *to* V. Compound XV (0·154 g) was suspended in a mixture of dioxane, water and sulphuric acid (3 ml; 35:65:2 by vol). The reaction was carried out as previously described for the hydrolysis of IX to V and 0·1 g of crude V were obtained (m.p. 130–133°; 69·4%). After recrystallization the product was identical with V obtained by hydrolysis of IX.

Methylether XV *from* XVI. Compound XVI (0·2 g) in 0·1 N HCl and methanol (25 ml) was allowed to stand at room temp for 24 hr. The solution was treated with excess silver carbonate, filtered and the solvent evaporated under reduced press. The residue was dissolved in CHCl; and

treated with charcoal to remove traces of colloidal silver. The solvent was evaporated again under reduced press and the residue (0·105 g) dissolved in benzene-pet ether (1 : 1) and passed through neutral alumina (activity III). After an oily fraction (0·017 g) a crystalline fraction (0·020 g) was separated by elution with benzene and finally 0·020 g of an oily product were obtained by elution with ethylether. The crystalline product, m.p. 110–112° was identical with XV.

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