# Terpenoids. LXX.[1] The Structure of the Sea Cucumber Sapogenin Holotoxinogenin

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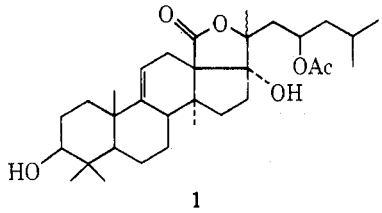
A new triterpenoid aglycone holotoxinogenin (**4a**) and its 25-methyl ether **5a** were isolated from the antifungal saponin holotoxin. The structure **4a** was shown to be 3β,20ξ,25-trihydroxy-16-oxolanost-9(11)-ene-18-carboxylic acid lactone (18 → 20). The ketonic functionality at C(16) is unprecedented in sapogenins from sea cucumbers. The full structure and stereochemistry was determined by X-ray analysis, thus establishing for the first time the absolute configuration of C(20) in the sea cucumber aglycones. Holotoxinogenin was found to be identical with stichopogenin $A_4$, whose earlier structure assignment is thus shown to be incorrect.

Sea cucumbers (Holothurians), members of the phylum Echinodermata, are known to possess toxic saponins in their body walls, as well as in the Cuvierian glands, for defensive and offensive purposes.[4] The toxicological and pharmacological aspects of these toxins have been studied extensively.[5] The chemical investigation of these saponins and their derived sapogenins began more than two decades ago and the tetracyclic triterpenoid structures of many of these compounds have since been established.[6,7] Recently, we reported the isolation and structure elucidation of a new sapogenin (1) from the dried skin of *Stichopus chloronotus* Brandt,[8] which contained only an isolated $\Delta^{9(11)}$-double bond without a C(12) substituent. Also noteworthy is the existence of a 23-acetoxy function.



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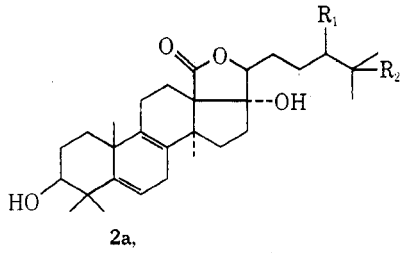
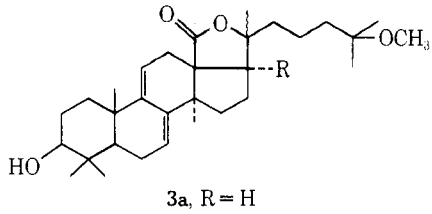


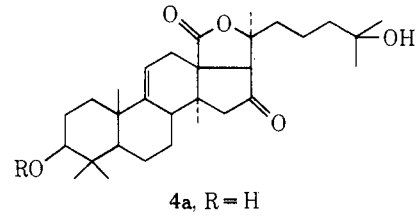
**2a**, b, $R_1 = H$; $R_2 = OH$



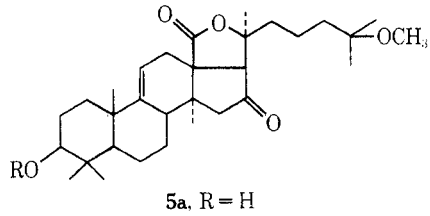
**3a**, R = H
b, R = OH

In 1969 Shimada reported the isolation of a new saponin, named holotoxin, from the body wall of the sea cucumber *Stichopus japonicus*,[9] the same species from which Elyakov, *et al.*,[10] isolated stichopogenins $A_2$ and $A_4$ and to which they assigned structures **2a** and **2b**. Crystalline holotoxin shows high activity against pathogenic fungi, a property that distinguishes it from the holothurins studied so far. The infrared spectrum of holotoxin is similar to that of holothurin but holotoxin lacks the sulfate group of the former. It is interesting to note that a considerable fraction of the biological potency of holothurin is linked to the possession of a negative charge center, and removal of this anionic character, *i.e.*, the sulfate group, causes a sharp decrease in its ability to destroy membrane excitability.[5a]

We report here the structures of two new sapogenins derived by acid hydrolysis of Shimada's holotoxin.[9] Due to the limited amount of material, the structures of holotoxinogenin (**4a**) and holotoxinogenin 25-methyl ether (**5a**)



**4a**, R = H
b, R = Ac



**5a**, R = H
b, R = Ac

were established predominantly by analysis of their spectral data and by X-ray analysis. Like 1[8] these genins contain the unusual 9(11) double bond system; in addition they are unique in having a ketone function at C(16).

The mass spectrum of compound **5a** depicted a molecular ion at *m/e* 500. The composition of **5a** as $C_{31}H_{48}O_5$ was established by high resolution measurements on fragment ions corresponding to the loss of methyl and methanol. The nature of the oxygen functions was based on the following observations.

The infrared spectrum of sapogenin **5a** revealed the presence of a five-membered lactone ($\nu_{C=O}$ 1755 $cm^{-1}$) and one hydroxyl group ($\nu_{OH}$ 3440 $cm^{-1}$) which could be acetylated. The secondary nature of this hydroxyl group was established by the presence of only one H–C–O type proton in **5a** and its derivatives. The strongly negative Cotton effect, $[\theta]_{303}$ −16,500, in the CD spectrum of **5a** indicated the presence of a carbonyl chromophore. Since the ir spectrum did not have absorption corresponding to either an open-

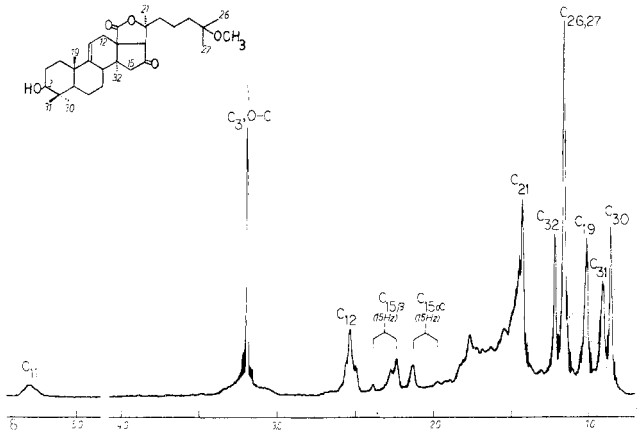


**Figure 1.** Nmr spectrum (100 Hz) of holotoxinogenin 25-methyl ether (**5a**) in deuteriochloroform.

chain ketone or a six-membered ring ketone, but showed broad absorption at 1755 $cm^{-1}$ the Cotton effect was presumably associated with a ketone contained in a five-membered ring. The negative Cotton effect would then require that it be either a 14$\alpha$-16-ketone or a 14$\beta$-15-ketone.[11] This point was settled rigorously by the X-ray data discussed below.

The nmr spectrum (Figure 1) of **5a** showed the presence of seven methyl groups in addition to a methoxyl function. The general resemblance with previously reported spectra[6-8,12,13] strongly indicated the presence of the typical lanostane skeleton with a $\gamma$-lactone between C(18) and C(20).

The $\beta$ configuration of the C(3) hydroxyl group was indicated by the position of a broad nmr absorption ($\delta$ 3.18) in the alcohol **5a**.[14] The $\beta$ configuration of the C(3) alcohol is further substantiated by the well documented[15] larger lanthanide induced shift of the 4$\beta$-methyl as compared to the 4$\alpha$-methyl group.[2]

The position of the methoxyl group at C(25) was established by the fact that the nmr signal of the C(26) and C(27) protons at $\delta$ 1.16 was similar to that of ternaygenin (**3a**)[12] and praslinogenin (**3b**).[13] The presence of the mass spectral peak at *m/e* 73 [$(CH_3)_2C{=}O^+CH_3$] confirmed that like **3a** and **3b**, compound **5a** contained a methoxyl function at C(25).

The nmr spectrum (Figure 1) of **5a** showed simple geminal coupling ($J$ = 15 Hz) at $\delta$ 2.05 and 2.31, which could be the result of the methylene protons $\alpha$ to a carbonyl group. This and the fact that the protons of the C(14) methyl group were more deshielded ($\delta$ 1.21) when compared with the C(14) methyl groups of praslinogenin (**3b**)[13] ($\delta$ 1.16) and ternaygenin (**3a**)[12] ($\delta$ 1.01) strongly suggest that the five-membered ring ketone function was at C(16).

The empirical formula of **5a** established by mass spectrometry required one more degree of unsaturation. There was only one olefinic proton ($\delta$ 5.3) in the nmr spectra (see Figure 1) of **5a** and **5b**. From spin decoupling data we concluded that the two-proton signal at 2.53 ppm was due to two allylic protons, which was consistent with three possible positions ($\Delta^5$, $\Delta^7$, or $\Delta^{9(11)}$, for the double bond. A definite conclusion was reached by X-ray crystallography.

The complete three-dimensional structure of holotoxinogenin 25-methyl ether (**5a**) was elucidated by a single crystal X-ray diffraction experiment. The results of this are shown in a drawing of the final X-ray model given in Figure 2. The X-ray work established firmly the 9–11 position of the double bond (1.35 Å distance) which could not be settled by the nmr studies; similarly it proved rigorously the

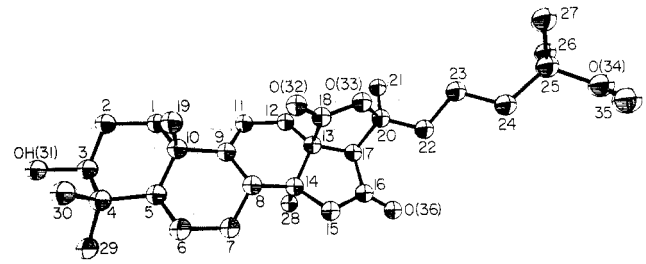
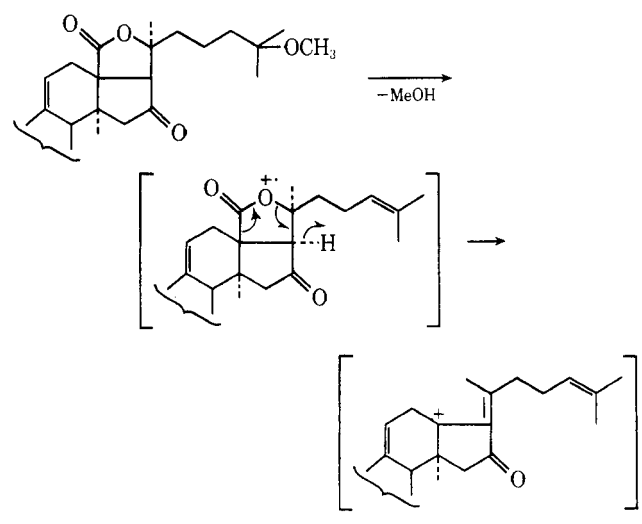


**Figure 2.** A computer generated perspective drawing of holotoxinogenin 25-methyl ether (**5a**). Atoms are carbons unless otherwise specified and hydrogens are not shown.

presence of the 16-keto group whose infrared signal was masked by the lactone absorption. The negative Cotton effect, mentioned previously, thus settles the absolute configuration of the molecule. In general, the bond distances were normal, the only abnormally short intermolecular contact being a hydrogen bond between O(31) and O(34) of 2.93 Å.

The diagnostic peaks in the mass spectra of **5a** and **5b** are summarized in Table I. The fragmentations could be rationalized readily in terms of structure **5**. Both compounds possess an intense peak at *m/e* 73.065 ($C_4H_9O$) corresponding to $(CH_3)_2C{=}O^+CH_3$ which is a characteristic fragment ion for sapogenins with a C(25) methoxyl group.[10b,12,13] The ion of mass 381.244 ($C_{25}H_{33}O_3$) originates from cleavage between carbon atoms 1 and 2 and 4 and 5, respectively, of ring A. When **5a** is acetylated, the mass spectrum of the monoacetate **5b** also contains an *m/e* 381 peak, indicating that the fragmentation is indeed the result of cleavage of ring A. The spectra of **5a** and **5b** display peaks at *m/e* 423 and 465, respectively, which could be generated by the loss of methanol, carbon dioxide, and a hydrogen atom. A conceivable mechanism is the following.



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In view of the fact that none of the previously known holothurinogenins exhibit such fragmentation, it seems reasonable to conclude that the presence of the C(16) keto group facilitates this fragmentation process by furnishing the $\alpha,\beta$-unsaturated ketone moiety of ion a.

The structure of holotoxinogenin (**4a**) was established by a comparison of its spectra with those of **5a**. The mass spectrum of **4a** showed a molecular ion peak at *m/e* 486 ($C_{30}H_{46}O_5$). Like its methyl ether **5a**, **4a** contained a $\gamma$-lactone ring ($\nu_{C=O}$ 1755 $cm^{-1}$), one or more hydroxyl groups ($\nu_{OH}$ 3440 $cm^{-1}$), and essentially no uv absorption above 210 nm. It also had a negative Cotton effect curve that was identical in shape and sign with **5a**. Acetylation of **4a** at room temperature gave a monoacetate (**4a**) whose ir spec-

**Table I**
**Diagnostic Peaks in the Mass Spectra of Holotoxinogenin 25-Methyl Ether (5a) and Its Acetate (5b)**

| | Relative intensity in % | |
|---|---|---|
| | 5a | 5b |
| $M^+$ | 500 (3) | 542 (1%) |
| $M - CH_3$ | 485 (7%) | 527 (6%) |
| $M - CH_3OH$ | 468 (100%) | 510 (97%) |
| $M - CH_3OH + CH_3$ | 453 (15%) | 495 (6%) |
| $M - CH_3OH + ROH^a$ | 450 (9%) | 450 (6%) |
| $M - CH_3OH + ROH^a + CH_3$ | 435 (15%) | 435 (26%) |
| $M - CH_3OH + CO_2 + H$ | 423 (12%) | 465 (10%) |
| $M - CH_3OH$ + ring A | 381 (4%) | 381 (2%) |
| $M - ROH^a$ + side chain | 367 (3%) | 367 (2%) |
| $(CH_3)_2C{=}O^+CH_3$ | 73 (57%) | 73 (100%) |

[a] RO refer to the 3β-OH or 3β-OAc substituent.

with that of $A_4$ except that it lacks the 25-hydroxyl group and contains instead a $\Delta^{24(25)}$ double bond.

Roller, *et al.*,[12] suggested that the methoxyl function at C(25) of **3a** and **3b** might be an artifact produced from a hydroxyl precursor during the acid hydrolysis conditions. It is likely that this has also happened here and that the naturally derived aglycone is holotoxinogenin (**4a**).

## Experimental Section

Melting points are uncorrected and were determined on a Kofler hot-stage microscope. All optical rotations were determined using chloroform as solvent. Infrared spectra were measured using a Perkin-Elmer Model 421 infrared spectrophotometer using polystyrene as external reference (1601 $cm^{-1}$). Ultraviolet spectra were measured in 95% ethanol on a Cary-14 recording spectrophotometer. Nuclear magnetic resonance (nmr) spectra were recorded on a Varian HA-100 or XL-100 spectrometer using deuteriochloroform as solvent. Tetramethylsilane was used as internal reference and line positions are given in the δ scale. Low-resolution mass spectra (70 eV) were obtained on AEI MS-9, Atlas CH-4, and Varian MAT-711 instruments with direct inlet systems. High-resolution spectra were determined on MS-9 and MAT-711 instruments.

Gas–liquid chromatography (glpc) was carried out on a Hewlett-Packard 402 high-efficiency instrument with glass columns packed with 3% of OV-25 on Gas-Chrom Q (100–200 mesh) from Applied Science Laboratories, Inc. Column chromatography was carried out using E. Merck neutral, activity grade II, aluminum oxide. Analytical scale thin layer chromatography was carried out on 5 × 20 cm, 250-μ silica gel $HF_{254}$ plates. Substances were visualized on these plates by spraying with ceric sulfate solution (2% in 1 *M* sulfuric acid) followed by heating on a hot plate.

We thank Dr. L. J. Durham for the nmr spectra, Mr. R. Ross, Mr. R. Conover, and Miss A. Wegmann for the mass spectra, and Mrs. R. Records for the CD measurements.

**Hydrolysis of Holotoxin.** Holotoxin[9,17] (1 g) was dissolved in 20 ml of 30% hydrochloric acid in methanol and heated under reflux for 3 hr. The mixture was diluted with methanol, the products precipitated by addition of water, and the aglycones extracted with dichloromethane. The dichloromethane layer was washed with water and sodium bicarbonate, dried (magnesium sulfate), and evaporated to give 340 mg of semisolid. Glpc demonstrated that it was a complex mixture of at least seven aglycones. The two major components holotoxinogenin (**4a**) and its 25-methyl ether (**5a**) were isolated in the following manner.

A 325-mg aliquot was dissolved in dichloromethane, absorbed on alumina activity II powder, and placed on an alumina II (50 g) column. Chromatography using gradient elution with benzene–ethyl acetate and several recrystallizations gave 39 mg of holotoxinogenin (**4a**) and 57 mg of holotoxinogenin 25-methyl ether (**5a**), both in about 90% purity (by glpc).

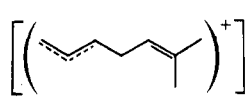
**Holotoxinogenin (4a):** mp 238–241° (from $CHCl_3$); $[\alpha]^{20}D$ −97.6° (*c* 0.25); CD (methanol) $[\theta]_{303}$ −14,274; ir (KBr) 3445 (broad, OH), 1750 (lactone C=O), 1460, 1440 (methylene adjacent to C=O), 1375, 1360, 1180, 1155, 1095, 1025, 940 $cm^{-1}$; essentially no uv absorption above 210 nm; nmr δ 0.86 (3, s, C(4α) $CH_3$), 0.91 (3, s, C(4β) $CH_3$), 1.01 (3, s, C(10) $CH_3$), 1.23 (9, s, C(14), C(25) $CH_3$), 1.42 (3, s, C(20) $CH_3$), 2.05 (1, one-half of AB quartet, $J$ = 15 Hz, C(15) H), 2.31 (1, one-half of AB quartet, $J$ = 15 Hz, C(15) H), 2.53 (1, broad, C(12) $H_2$), 3.19 (1, broad, C(3) H), 5.29 (1, broad, C(11) H); mass spectrum (relative intensity) 486 (4, $M^+$), 471,30811 (9, $M - CH_3$), 468.32153 (100, $M - H_2O$), 453.29980 (15, $M - H_2O + CH_3$), 450.31421 (9, $M - H_2O + H_2O$), 435.29053 (22, $M - H_2O + H_2O + CH_3$), 423.32397 (16, $M - H_2O + CO_2 + H$), 381.24268 (6, $M - H_2O$ + ring A), 367.22656 (5, $M - H_2O$ + side

**Table II**
**Values for Methyl Group Signals in Holotoxinogenin Derivatives**

| Compd | $C_4$–$CH_3$ | $C_{10}$–$CH_3$ | $C_{14}$–$CH_3$ | $C_{20}$–$CH_3$ | $C_{25}$–$CH_3$ | O–$CH_3$ | O–C(O)$CH_3$ |
|---|---|---|---|---|---|---|---|
| 4a | 0.86, 0.91 | 1.01 | 1.23 | 1.42 | 1.23, 1.23 | | |
| 5a | 0.85, 0.91 | 1.01 | 1.21 | 1.42 | 1.15, 1.15 | 3.18 | |
| 4b | 0.91, 0.92 | 1.15 | 1.25 | 1.42 | 1.23, 1.23 | | 2.08 |
| 5b | 0.90, 0.92 | 1.15 | 1.23 | 1.42 | 1.15, 1.15 | 3.18 | 2.08 |

trum still indicated a hydroxyl absorption. Thus the five oxygen atoms of **4a** were contained in a γ-lactone, a ketone, and two hydroxyl groups, one of them being nonacetylable. Further confirmation was provided by comparing the nmr spectra of **4a** and **4b** with those of **5a** and **5b** (Table II), which clearly show that **4a** differs from **5a** only in the functionality at position C(25). Since **4a** contains one $CH_2$ unit less than **5a** and has no methoxyl singlet, the substituent at C(25) has to be a hydroxyl, which would result in larger deshielding of the C(26) and C(27) protons.

The mass spectrum of **4a** showed peaks at *m/e* 471.308 ($C_{29}H_{43}O_5$, $M - CH_3$) and a base peak at *m/e* 468.322 ($C_{30}H_{44}O_5$, $M - H_2O$) and also ions due to subsequent loss of methyl and water from the fragment of mass 468. Like **5a**, **4a** also had a peak at *m/e* 423.324 ($C_{29}H_{43}O_2$) presumably generated by the 16-ketone function. The peak at *m/e* 73 $[(CH_3)_2C{=}O^+CH_3]$ was absent from the spectrum. Instead, intense ions at *m/e* 69,0704 $[(CH_3)_2C{=}CHCH_2^+]$ and *m/e* 109.602



were encountered. These fragments have also been observed in other sapogenins (*e.g.*, koellikerigenin[14]) which contain a C(25) hydroxyl group. It follows that holotoxinogenin has structure **4a**.

The fact that Elyakov's stichopogenin $A_4$ (**2b**) was also isolated from *Stichopus japonicus*, and possessed the same empirical composition and melting point as **4a**, prompted us to question the correctness of the unprecedented diene formulation of ring B (**2**). A direct comparison[16] (mixture melting point, CD, ir, glpc, and mass spectrum) of the monoacetates of stichopogenin $A_4$ (**2b**) and holotoxinogenin (**4a**) showed that they were identical in all respects and that the diene system (*cf.* **2**) was based on false assumptions. Examination of stichopogenin $A_2$ (**2a**) acetate indicated that it also contained a 16-keto group (from CD and mass spectral results) and hence its structure is identical

**Table III**
**Fractional Coordinates for Nonhydrogen Atoms of Holotoxinogenin 25-Methyl Ether[a]**

| Atom | x | y | z |
|---|---|---|---|
| C(1) | 1.0327 (3) | 0.416 (1) | 0.495 (1) |
| C(2) | 0.9480 (3) | 0.327 (1) | 0.477 (1) |
| C(3) | 0.9162 (3) | 0.264 (1) | 0.258 (1) |
| C(4)[b] | 0.9626 | 0.070 | 0.9655 |
| C(5) | 1.0496 (3) | 0.153 (1) | 0.1245 (9) |
| C(6) | 1.1023 (4) | −0.000 (1) | −0.032 (1) |
| C(7) | 1.1785 (4) | 0.121 (1) | −0.030 (1) |
| C(8) | 1.2171 (3) | 0.225 (1) | 0.1707 (9) |
| C(9) | 1.1661 (3) | 0.350 (1) | 0.3459 (9) |
| C(10) | 1.0849 (3) | 0.241 (1) | 0.3456 (9) |
| C(11) | 1.1945 (3) | 0.531 (1) | 0.512 (1) |
| C(12) | 1.2751 (3) | 0.628 (1) | 0.5373 (9) |
| C(13) | 1.3294 (3) | 0.454 (1) | 0.3926 (9) |
| C(14) | 1.2931 (3) | 0.353 (1) | 0.1724 (9) |
| C(15) | 1.3590 (4) | 0.210 (1) | 0.046 (1) |
| C(16) | 1.4289 (4) | 0.371 (1) | 0.136 (1) |
| C(17) | 1.4130 (3) | 0.524 (1) | 0.3612 (9) |
| C(18) | 1.3438 (4) | 0.274 (1) | 0.4857 (9) |
| C(19) | 1.0983 (4) | 0.042 (1) | 0.419 (1) |
| C(20) | 1.4631 (3) | 0.474 (1) | 0.523 (1) |
| C(21) | 1.4689 (4) | 0.682 (1) | 0.726 (1) |
| C(22) | 1.5388 (4) | 0.373 (1) | 0.445 (1) |
| C(23) | 1.5878 (4) | 0.324 (1) | 0.607 (1) |
| C(24) | 1.6561 (4) | 0.183 (1) | 0.511 (1) |
| C(25) | 1.7141 (4) | 0.152 (1) | 0.659 (1) |
| C(26) | 1.7530 (4) | 0.385 (1) | 0.792 (1) |
| C(27) | 1.6792 (4) | 0.039 (2) | 0.795 (1) |
| C(28) | 1.2808 (4) | 0.546 (1) | 0.099 (1) |
| C(29) | 0.9341 (4) | 0.053 (2) | −0.114 (1) |
| C(30) | 0.9469 (4) | −0.158 (1) | 0.114 (1) |
| O(31) | 0.8367 (2) | 0.204 (1) | 0.2460 (9) |
| O(32) | 1.2987 (3) | 0.1361 (9) | 0.5069 (8) |
| O(33) | 1.4164 (2) | 0.2927 (9) | 0.5562 (7) |
| O(34) | 1.7772 (2) | 0.0207 (9) | 0.5456 (8) |
| C(35) | 1.7610 (5) | −0.213 (2) | 0.431 (2) |
| O(36) | 1.4868 (2) | 0.380 (1) | 0.0474 (8) |

[a] The estimated standard deviation of the least-significant figure is given in parentheses. [b] Atom is used to define origin and never varied.

**Table IV**
**Bond Distances in Holotoxinogenin 25-Methyl Ether[a]**

| Atom pairs | Distance | Atom pairs | Distance |
|---|---|---|---|
| C(1)–C(2) | 1.54 | C(14)–C(28) | 1.52 |
| C(1)–C(10) | 1.53 | C(14)–C(15) | 1.54 |
| C(2)–C(3) | 1.53 | C(15)–C(16) | 1.52 |
| C(3)–O(31) | 1.41 | C(16)–O(36) | 1.20 |
| C(3)–C(4) | 1.56 | C(16)–C(17) | 1.53 |
| C(4)–C(29) | 1.51 | C(17)–C(13) | 1.54 |
| C(4)–C(30) | 1.53 | C(18)–O(32) | 1.20 |
| C(4)–C(5) | 1.57 | C(18)–O(33) | 1.34 |
| C(5)–C(10) | 1.55 | O(33)–C(20) | 1.46 |
| C(5)–C(6) | 1.50 | C(20)–C(17) | 1.57 |
| C(6)–C(7) | 1.51 | C(20)–C(21) | 1.52 |
| C(7)–C(8) | 1.47 | C(20)–C(22) | 1.49 |
| C(8)–C(9) | 1.49 | C(22)–C(23) | 1.56 |
| C(8)–C(14) | 1.51 | C(23)–C(24) | 1.51 |
| C(9)–C(10) | 1.54 | C(24)–C(25) | 1.53 |
| C(9)–C(11) | 1.35 | C(25)–C(26) | 1.53 |
| C(11)–C(12) | 1.49 | C(25)–C(27) | 1.52 |
| C(12)–C(13) | 1.53 | C(25)–O(34) | 1.44 |
| C(13)–C(14) | 1.55 | O(34)–C(35) | 1.43 |
| C(13)–C(18) | 1.53 | | |

[a] The estimated standard deviation is 0.01 Å.

chain, $C_6H_{13}O$), 328 (12), 274 (27), 259 (20), 241 (12), 109 (66), 69 (68), 55 (34), 43 (50).

**Holotoxinogenin 3β-Acetate (4b).** Holotoxinogenin (**4a**) (12 mg) was dissolved in 0.5 ml of (1:1) pyridine–acetic anhydride and stirred overnight. The usual work-up gave 13 mg of product. Recrystallization from methanol yielded 9 mg of **4b:** mp 221–223° (from MeOH); $[\alpha]^{20}D$ −84° (*c* 0.22); ir ($CHCl_3$) 3600 (OH), 1750 (lactone C=O), 1740 (five-membered ring C=O), 1720, 1240 (ester C=O), 1460, 1440 (methylene adjacent to C=O), 1360, 1150, 1125, 1090, 1025, 975, 940 $cm^{-1}$; nmr δ 0.91 (3, s, C(4α) $CH_3$), 0.92 (3, s, C(4β) $CH_3$), 1.15 (3, s, C(10) $CH_3$), 1.23 (6, s, C(25) $CH_3$), 1.25 (3, s, C(14) $CH_3$), 1.42 (2, s, C(20) $CH_3$), 2.08 (3, s, $CH_3$-C(O)-O), 2.05 (1, one-half of AB quartet, *J* = 15 Hz, C(15) H), 2.31 (1, one-half of AB quartet, *J* = 15 Hz, C(15) H), 2.53 (2, broad, C(12) $H_2$), 4.54 (1, broad, C(3) H), 5.19 (1, broad, C(11) H); mass spectrum *m/e* (relative intensity) 528 (2, $M^+$), 513 (4, M − $CH_3$), 510 (41, M − $H_2O$), 495 (4, M − $H_2O$ + $CH_3$), 450 (5, M − $H_2O$ + AcOH), 435 (21, M − $H_2O$ + AcOH + $CH_3$), 381 (3, M − $H_2O$ + ring A), 367 (3, M − AcOH + side chain), 465 (9, M − $H_2O$ + $CO_2$ + H), 328 (3), 316 (18), 241 (13), 109 (49), 69 (62), 55 (31), 43 (100).

**Holotoxinogenin 25-methyl ether (5a):** mp 236–238° (from $CHCl_3$) $[\alpha]^{20}D$ −125° (*c* 0.32); $CD_{methanol}$ $[\theta]_{303}$ −16,500; ir (KBr) 3440 (broad, OH), 1755 (lactone C=O), 1460, 1440 (methylene adjacent to C=O in five-membered ring ketone), 1380, 1362, 1185, 1095, 1080, 1025, 940 $cm^{-1}$; essentially no uv absorption above 210 nm; nmr δ 0.85 (3, s, C(4α) $CH_3$), 0.91 (3, s, C(4β) $CH_3$), 1.01 (3, s, C(10) $CH_3$), 1.15 (6, s, C(25) $CH_3$), 1.21 (3, s, C(14) $CH_3$), 1.42 (3, s, C(20) $CH_3$), 2.05 (1, one-half of AB quartet, *J* = 15 Hz, C(15) H), 2.31 (1, one-half of AB quartet, *J* = 15 Hz, C(15) H), 2.53 (2, broad, C(12) H), 3.18 (1, broad, C(3) H), 3.18 (3, s, $OCH_3$), 5.3 (1, broad, C(11) H); mass spectrum *m/e* (relative intensity) 500 (3, $M^+$), 485.32690 (12, M − $CH_3$), 468.32251 (100, M − $CH_3OH$), 453.2998 (15, M − $CH_3OH$ + $CH_3$), 450.31396 (9, M − $CH_3OH$ + $H_2O$), 435.2915 (15, M − $CH_3OH$ + $H_2O$ + $CH_3$), 423.32495 (11, M − $CH_3OH$ + $CO_2$ + H), 381.24390 (4, M − $CH_3OH$ + ring A), 367.22827 (3, M − $H_2O$ + side chain $C_7H_{15}O$), 328 (8), 274 (16), 259 (16), 241 (7), 231 (24), 109 (40), 73 (60), 55 (48), 43 (68).

**Holotoxinogenin 25-Methyl Ether 3β-Acetate (5b).** The holotoxinogenin 25-methyl ether (**5a**) (25.3 mg) was treated with 1:1 pyridine–acetic anhydride and set aside overnight at room temperature. The mixture was heated on a steam bath for half an hour and worked up in the usual way to yield 24 mg of product. Recrystallization from methanol gave 22 mg of **5b:** mp 230–233°; $[\alpha]^{20}D$ −71.5° (*c* 0.33); ir ($CHCl_3$) 1755 (lactone C=O), 1710, 1240 (ester –C(O) –O), 1740 (C=O in five-membered ring ketone), 1460, 1440 (methylene adjacent to C=O in five-membered ring ketone), 1376, 1362, 1095, 1085, 1025, 975, 940 $cm^{-1}$; nmr 0.90 (3, s, C(4α) $CH_3$), 0.92 (3, s, C(4β) $CH_3$), 1.15 (9, s, C(25) $CH_3$, C(10) $CH_3$), 1.23 (3, s, C(14) $CH_3$), 1.42 (3, s, C(20) $CH_3$), 2.05 (1, one-half of AB quartet, *J* = 15 Hz, C(15) H), 2.31 (1, one-half of AB quartet, *J* = 15 Hz, C(15) H), 2.08 (3, s, $CH_3$-C(O)-O), 2.53 (2, broad, C(12) $H_2$), 3.18 (3, s, $OCH_3$), 4.45 (1, m, C(3) H), 5.3 (1, broad, C(11) H); mass spectrum *m/e* (relative intensity) 542 (2, $M^+$), 527 (6, M − $CH_3$), 510 (97, M − $CH_3OH$), 495 (6, M − $CH_3OH$ + $CH_3$), 465 (10, M − $CH_3OH$ + $CO_2$ + H), 450 (5, M − $CH_3OH$ + AcOH), 435 (27, M − $CH_3OH$ + AcOH + $CH_3$), 381 (2, M − $CH_3OH$ + ring A), 367 (2, M − AcOH + side chain $C_7H_{15}O$), 328 (3), 316 (17), 241 (10), 226 (10), 124 (13), 109 (37), 84 (35), 73 (100), 69 (34), 55 (27), 43 (62).

**Crystallographic and X-Ray Data.** Holotoxinogenin 25-methyl ether (**5a**) crystallizes in the triclinic crystal system and since it is optically active the space group must be $P_1$. The following unit cell was chosen: *a* = 17.367 (4), *b* = 6.277 (5), *c* = 7.034 (3) Å, α = 112.78 (2), β = 89.79 (2), and γ = 92.25 (2)°. The cell constants and their associated errors were obtained from a least-squares analysis of 15 reflections with θ values between 30.0 and 40.0°. The calculated density was 1.17 g/$cm^3$ for one molecule of $C_{31}H_{47}O_5$ in the unit cell.

A crystal of dimension 0.35 × 0.26 × 0.09 mm was used for data collection. All unique reflections with θ ≤ 57° were collected on a fully automated four-circle Syntex $P2_1$ diffractometer. An ω scan of 2° was used because of the pronounced mosiac spread of the reflections. Backgrounds were collected on both sides of the scan for one-half of the scan time. A total of 1866 reflections were measured in this way. After correction for Lorentz polarization and

background effects 1601 reflections were judged observed, $|F_o| > 3\sigma(F_o)$. Three standard reflections were measured every hour and these showed no appreciable decline. The quantity $\sigma(F_o)$ was computed from $\{[l + \sigma(l)]/L_p\}^{1/2} - F_o$[18] and $\sigma(1)$ was computed from [total count + background count + 0.05(total count)$^2$ + 0.05(background)$^2$]$^{1/2}$.

**Determination and Refinement of Structure.** The observed structure factor amplitudes ($|F_o|^2$) were converted to normalized structure factors by removing the angular dependence of the reflections.[19] The solution of this 36 atom problem in the space group $P_1$ presented a severe challenge to direct methods. The largest 150 $E$'s ($E \geq 1.6$) were assigned phases using the multisolution tangent formula approach.[19] Of the resultant 32 solutions the one with the lowest $\psi_o$ residual was used to generate a Fourier map. The map was discouraging in that it resembled a continuous net of hexagons resembling chicken wire. The only encouraging aspect was the absence of outstandingly large peaks which meant that not much information had been destroyed by the squaring effect.[20] Nevertheless attempts to expand the model from various plausible fragments failed both through the use of the tangent formula[21] and through difference Fourier calculations using only those structure factors for which $F_c \geq 0.5F_o$. In both methods and for every fragment the model could not be forced to give additional atomic positions. Finally least-squares refinements with unit weights[22] did expand a 13-atom fragment into all 36 nonhydrogen atoms. Many cycles of least-squares refinements with anisotropic temperature factors for the nonhydrogen atoms and fixed-hydrogen atoms lowered the conventional discrepancy index to 0.048 for the 1601 observed reflections. Figure 2 is a computer generated perspective drawing of the final X-ray mode.[23] The absolute configuration is based on the negative Cotton effect CD measurement as only the relative configuration was determined by the diffraction experiment. (The estimated standard deviation in the bond lengths given in Table IV is 0.01 Å.) Table III is a listing of the fractional coordinates.

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**Registry No.**—**4a,** 53534-44-4; **4b,** 53534-45-5; **5a,** 53586-51-9; **5b,** 53534-46-6.

## References and Notes

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# Synthesis of Dipeptides of Aminophosphonic Acids[1]

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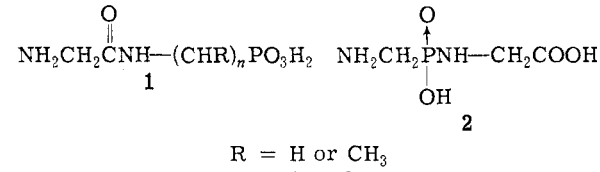
Of the two classes of phosphono dipeptide derivatives, three new parent dipeptides were synthesized using a new method followed by the removal of the protective groups and were characterized by elemental and detailed nmr analyses. In addition, for two dipeptides containing amide linkages and terminal phosphonic acid groups the p$K_a$'s and metal binding constants were determined. The peptides containing phosphonamide linkages could not be obtained in the free state because of their sensitivity toward acids and bases.

The recent isolation of 2-aminoethylphosphonic acid (2AEP) from several organisms[2a–e] and human beings[2f,g] has clearly shown that aminophosphonic acids are biologically an important class of compounds. Early publications of the natural occurrence of 2-aminoethylphosphonic acid suggested participation of the compound in lipid structures,[2a–e,3] but Quin[4] showed that occurrence in protein structures was also possible. Quin suggested[5] that the aminophosphonic acids could form part of polypeptide chains by amide formation through either one or both of their amino and phosphonic acid groups.

In a preliminary communication[6] we reported the preparation of the derivatives of several members of two classes (1 and 2) of phosphonic acid dipeptides. This note provides



1 2

R = H or $CH_3$
$n$ = 1 or 2

complete details for the removal of the protective groups; describes the isolation of three new dipeptides, glycyl-1- and 2-aminoethylphosphonic acid and glycylaminomethylphosphonic acid; provides nmr characterization; and reports the proto- and metallophilicity of the dipeptides acting as ligands. Furthermore, a much simpler route was