All other chemicals and solvents were analytical grade and were used without further purification.

5-[(β-Methyltelluro)ethyl]hydantoin (3). Sodium ditelluride (Na_2Te_2) was generated by reaction of tellurium powder (45 μm) with metallic sodium in liquid ammonia[10] and was alkylated with methyl iodide to give dimethyl ditelluride.[11] Sodium borohydride reduction[3] of the ditelluride (~11 mmol) in benzene-methanol (1:1) under an argon atmosphere generated methyltellurol. The 5-(β-bromoethyl)-hydantoin[13] (880 mg, 4.2 mmol) was added in a small volume of methanol, and the mixture was stirred at room temperature for 30 min, at which time TLC (system 1) indicated the reaction to be complete. The mixture was poured into water, and the aqueous solution was extracted with benzene, acidified to pH 2–3 with 10% H_2SO_4, and extracted with EtOAc. The desired product was extracted in the EtOAc layer, which was washed with water, dried over anhydrous Na_2SO_4, and evaporated to dryness in vacuo. The resulting tan-colored solid was crystallized from acetone–petroleum ether to give a white solid: 632 mg (55%); mp 132 °C; UV λ_{max} (MeOH) 232, 217 nm; IR ν_{max} (KBr) 1725, 1767 cm^{-1}; MS m/z 271.9815 (M^+; calculated for $C_6H_{10}O_2N_2Te$, 271.9820), 257 (M − CH_3), 186 (M − C_2O_2NH), 173 (M − hydantoin ring), 127 (M − CH_3Te), 99 (M − C_3H_7Te); ^1H NMR (Me_2SO-d_6, internal Me_4Si standard) δ 1.86 (s, 3 H, CH_3), ~1.94 (m, 2 H, β-CH_2), 2.56 (m, 2 H, γ-CH_2), 4.03 (m, 1 H, α-CH), 6.45 (s, 1 H, NH), 7.91 (s, 1 H, NH). The δ 6.45 and 7.91 resonances were absent in the spectrum of the sample shaken with D_2O.

DL-α-Amino-γ-(methyltelluro)butyric Acid (4c, "Telluromethionine"). The hydantoin 3 (40 mg, 0.15 mmol) was hydrolyzed with a 2 molar excess of 1 N NaOH in a teflon-lined bomb by heating at 165–167 °C for 1 h. The solution was carefully acidified to pH 5–6 with 10% H_2SO_4, filtered, and lyophilized to give a tan solid (4c and inorganic salts): UV λ_{max} (H_2O) 232, 217 nm; MS m/z 246.9879 (M^+; calculated for $C_5H_{11}NO_2Te$, 246.9881), 232 (M − CH_3), 202 (M − CO_2H), 186 (M − H_2O − CH_3 − CO), 173 (M − CO_2H − CH_3N), 74 (M − CH_3Te − H_2O), 56 (C_3H_6N); ^1H NMR (D_2O) (Figure 1).

A second sample of the hydantoin (0.15 mmol) was hydrolyzed in 1 N NaOD and worked up in the same manner: UV λ_{max} (H_2O) 232, 217 nm; MS m/z 248 (M^+), 233 (M − CH_3), 203 (M − CO_2H), 187 (M − H_2O − CH_3 − CO), 173 (M − CO_2H − CH_2DN), 75 (M − CH_3Te − H_2O), 57 (C_3M_5DN); ^1H NMR (D_2O, internal, 2,2-dimethyl-2-silapentane-5-sulfonate standard) δ 1.88 (s, 3 H, CH_3), 1.99 (m, 2 H, β-CH_2), and 2.57 (m, 2 H, γ-CH_2).

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Registry No.—1, 25284-83-7; 2, 68876-71-1; 3, 68876-72-1; 4a, 63-68-3; 4b, 2578-28-1; 4c, 68876-73-3.

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Reinvestigation of the Structure of Ristomycinic Acid, a Bis(amino acid) Obtained from Ristomycin

Thomas M. Harris,* Constance M. Harris, and James R. Fehlner

Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37235

Rezso Bognár and Ferenc Sztaricskai

Research Group of Antibiotics, Hungarian Academy of Sciences and Institute of Organic Chemistry of L. Kossuth University, H-4010 Debrecen, Hungary

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Ristomycinic acid is a component of the peptide in ristomycin A, which is an antibiotic elaborated by *Proactinomyces fructiferi* var. *ristomycini*. The compound was assigned as 1 on the basis of the empirical formula $C_{17}H_{18}N_2O_8$ which was established by elemental analyses, NMR spectra, and other data, the most important being the structures of hydrogenolysis products.[1] This compound has been claimed[2] to be present in a similar antibiotic, ristocetin A, which is a metabolite of *Nocardia lurida*, but studies by Fehlner et al.[3] indicated a different empirical formula ($C_{17}H_{18}N_2O_7$) for the corresponding substance from that source. Harris et al.[4] assigned structure 2 to the compound from ristocetin on the basis of oxidative degradation to esters 3 and 4, followed by an independent synthesis of 4. A recent investigation of the mass spectrum of the dimethyl ester of tri-*O*-acetyl-*N*,*N*′-diacetylristomycinic acid by Katrukha et al.[5] showed that unprotected ristomycinic acid has the empirical formula $C_{17}H_{18}N_2O_7$, identical with that assigned by Fehlner et al.[3] for the compound from ristocetin. On the basis of this spectrum, Katrukha concluded that the compounds from ristomycin A and ristocetin A appear to be identical, but the data do not exclude the possibility that ristomycinic acid and the compound from ristocetin are structural isomers with similar chemical and chromatographic behavior. We now wish to report our studies of ristomycinic acid which establish 2 as its structure.

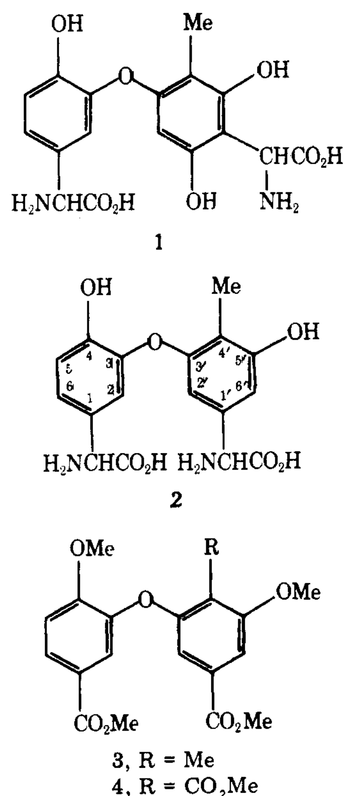

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Table I. ^{1}H NMR Spectrum of Ristomycinic Acid[a]

signal, δ	mult., area	assignment
2.23	s, 3 H	C-Me
5.17	s, 1 H	α-CH
5.20	s, 1 H	α-CH
6.72	d, J = 2 Hz, 1 H	2′- or 6′-H
7.00	d, J = 2 Hz, 1 H	6′- or 2′-H
7.05	broadened s, 1 H	2-H
7.30	broadened s, 2 H	5- and 6-H

[a] D_2O solution; spectrum was calibrated against internal MeOH (δ 3.47).

Table II. ^{13}C NMR Spectrum of Ristomycinic Acid[a]

signal, δ	mult.	assignment	signal, δ	mult.	assignment
9.2	q	CH_3	125.6	s	1-C
57.1	d	α-CH	131.5	s	1′-C
57.2	d	α-CH	145.8	s	4-C
110.8	d	2′- or 6′-C	149.0	s	3-C
111.6	d	6′- or 2′-C	156.7	s	5′- or 3′-C
118.7	d	5-C	157.1	s	3′- or 5′-C
119.5	s	4′-C	171.6	s	C=O
119.6	d	2-C	171.8	s	C=O
125.1	d	6-C			

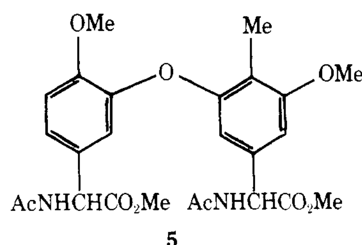
[a] D_2O solution; spectrum was calibrated against internal MeOH (δ 50.0).

The ^{1}H NMR spectrum of ristomycinic acid in D_2O (Table I) is essentially identical with that of **2** from ristocetin, but could not be used with certainty to exclude other isomers.[6] The spectrum shows the presence of five aromatic protons grouped 2:2:1, in support of **2** or isomers thereof. Lomakina's NMR study failed to yield an integral of sufficient accuracy to establish this point, structure **1** having only four aromatic protons.[2] Interpretation of the ^{1}H NMR spectrum is complicated by protons 5 and 6 having the same chemical shifts and by partial overlap between the signals for proton 2 and one of the protons on the other ring. The chemical shifts of the three downfield aromatic protons are consistent with a 3,4-dioxy substituted phenylglycine.

Because of the increased resolution, ^{13}C NMR spectra provided definitive evidence for the two bis(amino acids) having the same structure. The spectrum of ristomycin-derived material is listed in Table II;[6] multiplets resulting from one-bond proton coupling are indicated. The assignments of resonances were made by the usual methods;[7] comparison with spectra of other substituted phenylglycines aided these assignments. Several ambiguities were resolved using undecoupled spectra, which served to identify meta or other three-bond relationships with protons ($^3J_{CCCH}$ = 4–7 Hz).

In order to obtain chemical evidence for ristomycinic acid being **2**, it was converted to the O-methylated amide ester by N-acetylation with acetic anhydride and then by methylation with dimethyl sulfate followed by diazomethane. The mass spectrum of this compound showed a parent ion at m/e 502 in support of formulation **2** for the free amino acid and in confirmation of Katrukha's measurement.[5] The ^{1}H NMR spectrum showed the presence of one C-methyl, two N-acetyl, and four O-methyl groups (two esters and two ethers). The aromatic pattern was similar to that of the free amino acid, but provided no assistance in distinguishing between the various possible isomers. The spectrum was identical with that of **5** obtained from ristocetin.[8] On TLC the compound had the same retention as the more mobile diastereoisomer of **5**, which is believed to be the one having the native configuration.

The protected amide ester was hydrolyzed in acid; the resulting bis(amino acid) was converted to diester **3** by treatment with (1) excess NaOCl under alkaline conditions, (2) hydrolysis in refluxing KOH, and (3) esterification with CH_2N_2. The diester obtained was identical in all respects with diester **3** obtained from a similar degradative sequence on **2** from ristocetin;[4] ^{1}H NMR spectra were of particular importance in this comparison. The structure of **3** has been established by an independent synthesis involving Ullmann condensation between methyl 3-hydroxy-5-methoxy-p-toluate and methyl 3-bromoanisate.[8]



Experimental Section

^{1}H NMR spectra were recorded with a JEOL MH-100 NMR spectrometer at 100 MHz. Mass spectra were obtained with an LKB 9000A mass spectrometer by direct insertion. High pressure liquid chromatography was performed on a Waters Associates instrument equipped with a UV detector; a 2-ft μ-Porasil column was used. Merck precoated silica gel 60 F-254 plates were used for TLC with detection by UV and exposure to I_2.

Preparation of 5. Ristomycinic acid (26.2 mg), dissolved in 2 mL of 1 M $NaHCO_3$, was treated with 100 μL of acetic anhydride added in four portions over 1 h while the temperature of the reaction mixture was gradually raised from 0 °C to room temperature. After 2 h, 2 mL of 2 M aqueous NH_4OH was added to hydrolyze any phenolic acetates that might have been formed. After 30 min, the solution was evaporated to dryness; the residue was dissolved in 2 mL of 1 M K_2CO_3 and treated with 50 μL of dimethyl sulfate. After 2 h at room temperature the reaction mixture still gave a positive test with diazotized benzidine, so additional dimethyl sulfate (25 μL) was added. After an additional 16 h, the reaction mixture was acidified and extracted with 9:1 ethyl acetate–methanol. The organic solution was dried and evaporated. The residue was dissolved in methanol and treated with diazomethane for 2 h. After evaporation of the solution, 33.6 mg of crude protected amide ester **5** was obtained; the mass spectrum indicated that it was primarily the desired compound, although a small amount of partially methylated material was present. Purification by LC (CH_2Cl_2–MeOH, 98:2) removed minor contaminants. The material which was isolated appeared to be identical with the more mobile diastereoisomer of **5** which had been isolated from ristocetin:[8] ^{1}H NMR ($CDCl_3$) δ 2.02 (s, 6 H, 2 × $COCH_3$), 2.14 (s, 3 H, C-Me), 3.67 (s, 3 H, OMe), 3.72 (s, 3 H, OMe), 3.88 (s, 6 H, 2 × OMe), 5.43 (d, 2 H, 2 × α-CH), 6.46–6.93 (m, 5 H, aryl); MS m/e 502 ($M^{+\cdot}$, 53%), 470 (63), 459 (100), 443 (33), 411 (33), 401 (83), 368 (13), 357 (6), 342 (33), 341 (50).

Preparation of Diester 3. Compound **5** (2.8 mg) was refluxed for 15 h with 1 N HCl (4 mL) to give the corresponding bis(amino acid): ^{1}H NMR (D_2O acidified) δ 2.09 (s, 3 H, C-Me), 3.84 (s, 6 H, 2 × OMe), 6.38–7.02 (m 5 H, aryl). The α-CH signals were obscured by the HDO peak. The compound, dissolved in 1 mL of 1 M KOH, was added dropwise to 1 mL of a solution of 0.35 M NaOCl in 0.45 M KOH at 0 °C. The mixture was allowed to warm to room temperature over 1 h. Excess NaOCl was destroyed with Na_2SO_3; 2 mL of 60% aqueous KOH was added. The solution was refluxed for 4 h, cooled, acidified, and extracted with EtOAc–MeOH (9:1). The extract was dried and evaporated. The diacid, dissolved in methanol, was treated with ethereal diazomethane for 30 min. The product was purified by TLC (ethyl acetate–pentane, 3:7) to give diester **3**, which was identical by TLC, NMR, and MS with **3** isolated from a similar sequence of reactions on ristocetin and synthesized by an independent route.[4,8]

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Registry No.—**2**, 54750-25-3; **3**, 57466-20-3; **5**, 68926-47-6.

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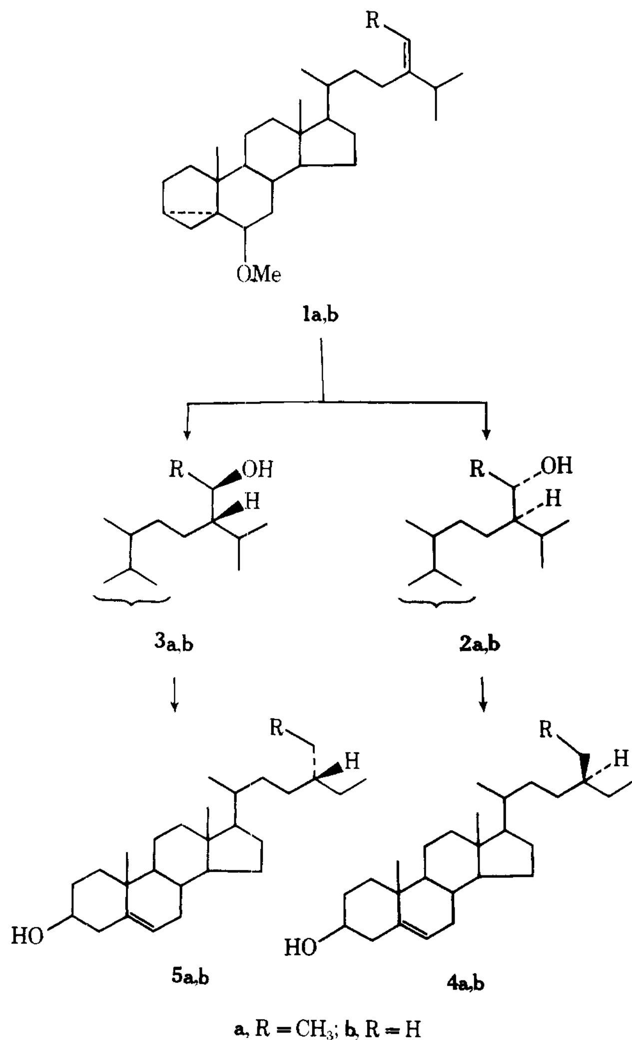
Convenient Preparation of the C-24 Stereoisomers of 24-Ethyl- and 24-Methylcholesterols[1]

Yoshinori Fujimoto and Nobuo Ikekawa*

Laboratory of Chemistry for Natural Products, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama, 227, Japan

Received September 11, 1978

Although syntheses of (24*R*)- and (24*S*)-alkylsterols have already been reported,[2,3] we have searched for another simple method of their preparation. The one described here allows the introduction of tritium or deuterium stereospecifically at C-24 and/or C-28 of 24-alkylsterols and may be useful for the stereochemical investigations of C-24(28) dehydrogenation, which is the first step of sterol metabolism in phytophagous insects.[4]



a, R = CH_3; **b**, R = H

Hydroboration–oxidation of the 3,5-cyclo derivative (**1a**) of fucosterol gave a diastereoisomeric mixture of 28-hydroxyl derivatives, which was resolved by column chromatography on silica gel. Elution with hexane–benzene (1:2.5) afforded the less polar isomer (**2a**), mp 115–116.5 °C, and further elution with hexane–benzene (1:3) gave the more polar isomer (**3a**). The configurations at C-28 of these isomers were determined to be 28*S* for the less polar compound and 28*R* for the more polar one by the modified method of Horeau using GLC.[5] These results suggested the configuration at the C-24 position to be 24*R* for the less polar and 24*S* for the more polar isomer in view of the established mechanism of hydroboration. The assignments were confirmed by the transformation of the two alcohols to the corresponding 24-ethylcholesterols. The 28-mesylates of **2a** or **3a** were reduced with $NaBH_4$–HMPA[6] to give the 3,5-cyclo derivatives of 24-ethylcholesterol (70%) accompanied by the $\Delta^{24(28)}$ analogues (30%), which were removed by epoxidation with *m*-chloroperbenzoic acid followed by silica gel chromatography. By treatment of the 3,5-cyclo derivative with acid, **2a** gave (24*R*)-24-ethylcholesterol (sitosterol) (**4a**), mp 136.5–138 °C (20% from **1a**), and **3a** gave (24*S*)-ethylcholesterol (clionasterol) (**5a**), mp 141–142.5 °C.

Hydroboration–oxidation of the 3,5-cyclo derivative of 24-methylenecholesterol (**1b**) afforded the less polar 28-ol (**2b**) and the more polar 28-ol (**3b**), which were separated from each other by preparative TLC, developing with benzene–ethyl acetate (25:1) four times. Reduction of both 28-mesylates with $LiAlH_4$ followed by acid treatment gave (24*R*)-24-methylcholesterol (campesterol) (**4b**), mp 160.5–161 °C (20% from **1b**), from the less polar 28-ol and (24*S*)-24-methylcholesterol (dihydrobrassicasterol) (**5b**), mp 158.5–160 °C (20% from **1b**), from the more polar one.

^{1}H NMR spectra (100 MHz) of the synthetic 24-ethyl and -methyl compounds are in good agreement with the published data.[7] ^{13}C NMR spectra of the C-24 isomers[8] were also found to be distinguishable from each other, and the spectra of **4a** and **4b** were identical with those of authentic sitosterol and campesterol, respectively. The signals of C-20, -21, -23, and -24 of the 24*R* isomers appear at higher field than those of the 24*S* isomers. Thus, ^{13}C NMR is also useful for the identification of C-24 alkylsterol isomers as ^{1}H NMR reported recently.[7]

Experimental Section

Melting points were determined on a hot stage microscope and are uncorrected. ^{1}H NMR spectra were recorded on a Hitachi R-24A (60 MHz) or a JOEL JNM-4H-100 (100 MHz) in $CDCl_3$ solution with Me_4Si as an internal standard. ^{13}C NMR spectra were determined with a JEOL PS/PFT-100 spectrometer at 25.2 MHz in $CDCl_3$. Mass spectra were recorded on a Shimadzu LKB-9000.

6β-Methoxy-3,5-cyclostigmast-24(28)-ene (1a). A mixture of 1.41 g of fucosterol, 40 mL of pyridine, and 1.4 g of *p*-toluenesulfonyl chloride was stirred at room temperature overnight. The reaction mixture was poured into cold water, and the resulting precipitate was collected by filtration, washed with water, and dried in vacuo. The tosylate (1.45 g) was used for the next step without purification. A mixture of 1.45 g of the tosylate, 200 mL of methanol, and 2.0 g of KOAc was refluxed for 3 h.[9] After most of the solvent was evaporated, the mixture was extracted with ethyl acetate. The organic layer was washed with saturated NaCl, dried over $MgSO_4$, and evapoarated. Chromatography (eluted with hexane) of the product on silica gel gave 1.05 g of **1a** as an oil: NMR (60 MHz) δ 0.2–0.7 (1 H, m, 3-H), 1.6 (3 H, d, J = 6.5 Hz, 29-Me), 2.75 (1 H, m, 6-H), 3.3 (3 H, s, MeO), 5.1 (1 H, q, J = 6.5 Hz, 28-H). Anal. Calcd for $C_{30}H_{50}O$: M^+ *m/e* 426.3861. Found: M^+ *m/e* 426.3871.

(24*R*,28*S*)- and (24*S*,28*R*)-6β-Methoxy-3,5-cyclostigmast-28-ol (2a and 3a). To a solution of 1.05 g of **1a** in 20 mL of dry THF was added 5 mL of BH_3–THF complex in benzene (~1 N) under a

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