Linustatin and Neolinustatin: Cyanogenic Glycosides of Linseed Meal That Protect Animals against Selenium Toxicity

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Two new cyanogenic glycosides—linustatin (**1a**) and neolinustatin (**1b**)—have been isolated from linseed meal. These glycosides are responsible for linseed's unique property of protecting animals against the toxic effects of ingested selenium. This investigation demonstrates the facility with which glycosidic structures can be elucidated by ^{13}C NMR spectroscopy.

For over 20 years, it has been recognized that the defatted meal from flaxseed (*Linum usitatissimum*) possesses unique properties in protecting livestock against the toxic effects of selenium compounds in forage plants.[1] Halverson and co-workers demonstrated that the protective principle could be removed from linseed meal by extraction with ethanol–water (1:1).[1] The early work of Halverson et al. now has culminated in the isolation and characterization of two compounds responsible for this protective action. These active substances are new cyanogenic glycosides for which we propose the trivial names linustatin [2-[(6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]-2-methylpropanenitrile] and neolinustatin [(2*R*)-[(6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]-2-methylbutanenitrile]. The present paper describes the structural elucidation of these glycosides; accounts of biological experiments with these compounds will be published elsewhere.[2]

Isolation of Glycosides. Guided by appropriate biological assays,[2] we fractionated the residue from the aqueous ethanolic extract of linseed meal in a series of steps involving extraction with water, with methanol, and with chloroform–methanol (2:1) and chromatography on a silicic acid column (see Experimental Section). The active chromatographic fraction was further separated by high-pressure liquid chromatography (LC) into three components, two of which proved to be active in the selenium-protection assay. These active compounds, with empirical formulas $C_{16}H_{27}NO_{11}$ (linustatin, **1a**) and $C_{17}H_{29}NO_{11}$ (neolinustatin, **1b**), were isolated as levorotatory crystalline solids.

Characterization of Glycosides. The IR spectra of **1a** and **1b** show very strong hydroxyl absorption (3400 cm^{-1}, br) and a small maximum attributable to C≡N (2240 cm^{-1}); no carbonyl absorptions are apparent. ^{13}C NMR spectra (Table I) reveal a series of signals indicating that **1a** and **1b** are cyclic polyols and are probably disaccharides derived from two aldopyranose units.[3] In the off-resonance-decoupled spectra, there are two series of doublets in the region δ 72–79 which are associated with carbons 2–5 and 2′–5′ of the two sugar units. The anomeric carbons, C-1 and C-1′, appear as doublets near δ 101 and 105.5. When **1a** and **1b** are completely hydrolyzed with mineral acid, the only monosaccharide produced is glucose.

Compilations of ^{13}C NMR data reveal certain characteristic chemical shifts for the various carbons of common sugars; these values provide a basis for differentiating both α and β anomers of these sugars, whether they are free or glycosidically bound.[3-5] Moreover, the work of Usui et al. documents differences that enable us to distinguish various glucobioses through ^{13}C NMR.[6] The observed shifts for linustatin and neolinustatin (Table I) mark them as closely related disaccharides composed of two glucopyranose units. The spectra of **1a** and **1b** show one triplet at δ 63.6 characteristic of an unsubstituted hydroxymethyl group at one C-6 position. The other C-6 triplet occurs considerably downfield at δ 71. Accordingly, the two glucose units must be attached through β-1,6 linkages, as in gentiobiose (**1c**), since an α-1,6 linkage, as in isomaltose, would require a C-6 resonance at δ 67.4; other types of glycosidic linkages (including 1,1, 1,2, 1,3, and 1,4) are excluded by the data of Usui et al.[6] Comparisons of ^{13}C NMR shifts for **1a**, **1b**, and **1c** reveal good agreement of values for carbons 2, 3, 4, and 5 in both rings. Within the confines of glucobiose groups, ^{13}C NMR shifts for **1a** and **1b** coincide uniquely with those of gentiobiose, except that for C-1, the anomeric center.

The structure of the aglycon moieties of **1a** and **1b** likewise can be deduced through their NMR spectra. For both compounds, the lowest field ^{13}C NMR signal is a singlet near δ 124, appropriately situated for a nitrile function.[7,8] The highest field signals in the ^{13}C NMR spectrum of **1a** (quartets at δ 28.8 and 29.8) together with the highest field proton resonances (three-proton singlets at δ 1.60 and 1.65 for the heptaacetate of **1a**) indicate two methyl groups insulated from other proton-bearing carbons. The complement of ^{13}C NMR signals for the aglycon portion of **1a** is completed by a δ 78.7 singlet due to the quaternary carbon. The corresponding spectra of **1b** and its heptaacetate reveal one methyl and one ethyl group in the aglycon moiety. The ethyl group is documented by a quartet at δ 10.8 and a triplet at δ 35.9; complementary proton signals appear as a triplet at δ 1.08 coupled with

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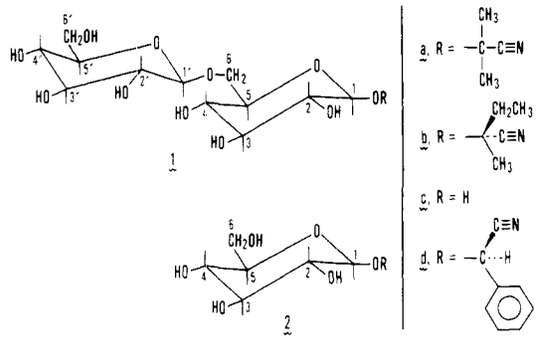
Table I. ^{13}C NMR Assignments for Linustatin, Neolinustatin, and Related Compounds[a]

carbon	β-gentiobiose (1c)[b]	linustatin (1a)	neolinustatin (1b)	amygdalin (1d)	linamarin (2a)	2a from hydrolysis	lotaustralin (2b)	2b from hydrolysis
1′	105.5	105.5, d	105.5, d	105.7				
2′	75.9	76.0, d	76.0, d	76.1				
3′	78.7	78.4, d	78.4, d	78.5				
4′	72.4	72.6, d	72.5, d	72.6				
5′	78.7	78.4, d	78.4, d	78.5				
6′	63.6	63.6, t	63.6, t	63.7				
1	98.8	101.6, d	101.3, d	104.4	101.7	101.8	101.4	101.4
2	76.8	75.6, d	75.6, d	75.6	75.6	75.7	75.6	75.7
3	78.7	78.7, d	78.7, d	78.8	78.9	79.0	78.9	79.0
4	72.4	72.1, d	72.1, d	72.1	72.3	72.3	72.3	72.3
5	77.7	78.2, d	78.1, d	78.3	78.3	78.4	78.4	78.4
6	71.6	71.1, t	71.1, t	71.2	63.4	63.5	63.4	63.4
pri or tert CH_3		29.8, q	10.8, q		29.7	29.6	10.6	10.7
quaternary C		74.7, s	78.7, s	71.9	74.8	74.9	78.6	78.6
tertiary CH_3		28.8, q	25.9, q		29.1	29.1	25.9	26.1
CH_2			35.9, t				35.7	35.8
C≡N		124.3, s	124.0, s	121.5	124.3	124.3	123.9	123.9
aromatic				130.6, 132.3, 133.6, 135.4				

[a] Samples were dissolved in D_2O. Chemical shifts (δ) are expressed in ppm from internal TSP ($Me_3SiCD_2CD_2CO_2Na$). Multiplicities of signals shown for **1a** and **1b** apply to all shifts in the same row except the benzylic carbon in **1d**. [b] Based on an equilibrated solution prepared from β-gentiobiose. Also observed were shifts due only to a α-gentiobiose: δ 93.3, 72.9, 74.5, 71.8.

Scheme I



a quartet at δ 1.88. These structural features indicate a close relationship between linustatin, neolinustatin, and two previously known cyanogenic glycosides—linamarin (**2a**) and lotaustralin (**2b**).[9,10] Comparisons of the ^{13}C NMR spectra of **2a** and **2b** with those of **1a** and **1b** (Table I) reinforce this conclusion through significant similarities. Shifts for carbons 1–5 are closely comparable in the spectra of **1a**, **1b**, **2a**, and **2b**; shifts for the aglycon carbons of **1a** and **2a** are in good agreement, as are those for **1b** and **2b**.

Amygdalin (**1d**), one of the more common cyanogenic glycosides in nature, is derived from β-gentiobiose; chemical shifts for carbons in the sugar moiety of **1d** are in striking agreement with those of **1a** and **1b** except the value for anomeric C-1. In ^1H NMR spectra of heptaacetates of **1a** and **1b**, the C-1 proton appears at δ 4.68 (d, J = 8 Hz), thus indicating an axial proton and confirming the β configuration of this glycosidic linkage.[11]

Hydrolysis of Linustatin and Neolinustatin. Results of enzymatic hydrolysis of **1a** and **1b** provided additional and conclusive proof for the structures in Scheme I. When treated with a β-glucosidase (almond emulsin), **1a** was cleaved regiospecifically into D-glucose (**2c**) and a degradation product identical with linamarin (**2a**) as determined by ^{13}C NMR (Table I), ORD, and melting points. When treated with the same enzyme, **1b** yielded glucose and a product identical with lotaustralin (**2b**). One might expect that a β-glucosidase would hydrolyze both β-glucosidic linkages in **1a** and **1b**; however, resistance of these particular C-1 linkages to almond emulsin has been noted previously.[12-14]

There appears to be no available regiospecific method, either enzymic or chemical, to cleave the C-1 glycosidic linkages of **1a** and **1b** and thus afford β-gentiobiose as a discrete product. Apparently, the 1,6-glycosidic linkage is more rapidly cleaved by available techniques than are most β-glucosidic bonds at C-1. However, we were able to detect β-gentiobiose (**1c**) as a minor product after partial hydrolysis of **1b** under mild acidic conditions along with **2b**, **2c**, unchanged **1b**, and three unidentified products.

Absolute Configuration of Linustatin and Neolinustatin. Enzymatic hydrolyses of **1a** and **1b** yielded products with ORD curves that are virtually identical with those of **2a** and **2b**, respectively. Accordingly, since sugar moieties of **1a** and **1b** contain two D-glucose units joined through β-glycosidic linkages, the absolute configurations of all the chiral centers of **1a** and **1b** are defined as in Scheme I. These conclusions are reinforced by the ORD spectrum of amygdalin (**1d**), which is similar in sign and magnitude to those of **1a** and **1b**. Moreover, the chiral center in the aglycon portion of neolinustatin (**1b**) must have the same configuration as that of lotaustralin (**2b**); according to Bissett et al., that configuration is *R*.[15] Zilg and Conn demonstrated that L-isoleucine [(3*S*)-L_s-isoleucine] and its diastereomer, (3*R*)-L_s-isoleucine, are incorporated into the aglycon moiety of lotaustralin or epilotaustralin and that the stereochemistry at C-3 of isoleucine is retained throughout the intermediate transformations.[16,17]

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Discussion

The title compounds of this paper take their places in a class of natural products whose numbers are rather restricted despite having been objects of interest and investigation for well over a century. Seigler's 1975 review lists only 20 cyanogenic glycosides with fully elucidated structures.[9]

In previous investigations of flax, the only cyanogenic glycosides encountered have been linamarin and lotaustralin, a pair which generally occur together.[14] Butler et al. reported a 55:45 mixture of **2a** and **2b** in flax seedlings.[14] In our isolation work, linseed meal was extracted with hot ethanol immediately after grinding; this treatment would be expected to inactivate any enzymes present, including β-glucosidases. In view of the facile degradation of **1a** and **1b** with almond emulsin, it is conceivable that at least portions of the **2a** and **2b** obtained by previous workers were artifacts of isolation if any β-glucosidases were active during the extraction steps. Our work was guided by a specific bioassay, and we did not encounter **2a** and **2b** during our fractionation; the disposal of these compounds, if present initially in our extract, was not determined.[18]

This investigation illustrates the exceptional facility with which the structures of glycosides can be elucidated by ^{13}C NMR if suitable reference compounds are available. In contrast, the ^1H NMR spectra of **1a** and **1b** were relatively uninformative, although they served to confirm certain features of the aglycon moieties. To our knowledge, the ^{13}C NMR spectrum of a cyanogenic glycoside has not been reported previously with complete assignment of shifts. However, observed ^{13}C shifts for acacipetalin[19] and amygdalin[20] have been recorded and partially assigned. ^1H NMR spectra of cyanogenic glycosides were discussed in Seigler's review;[9] more recently, Turczan and co-workers have published a detailed study of the 220-MHz ^1H NMR spectra of several compounds of this class.[21]

Experimental Section

General Procedures.[22] ^{13}C NMR spectra were determined with a Bruker WH-90 Fourier transform NMR spectrometer, operating at 22.63 MHz with proton-noise decoupling. The computer data memory size used for the real part of the spectra was 8K. The δ values are accurate to within ±0.1 ppm. A 5 μs (ca. 30°) pulse width was used. The spectra (2500–100 000 accumulations) were obtained from solutions in D_2O which also served as a deuterium lock. Chemical shifts are given as δ values in ppm downfield from internal TSP-^{13}C ($Me_3SiCD_2CD_2CO_2Na$). Multiplicity of the signals was determined by off-resonance partially decoupled spectra. ^1H NMR were determined with a Varian HA-100 instrument. IR spectra were determined with a Perkin-Elmer Model 521 (KBr pellets) or with a Perkin-Elmer Model 700 ($CHCl_3$ solutions) instrument. ORD spectra were recorded with a Cary Model 60 spectropolarimeter. LC was carried out with a Waters Model 6000 instrument (analytical separations) or a Waters System 500 instrument (preparative separations), both equipped with refractive index detectors. TLC analyses were carried out on plates spread with 0.25-mm layers of silica gel G (E. Merck, Darmstadt); components were visualized by charring with sulfuric acid or sulfuric acid–dichromate solution. Melting points were determined with a Fisher-Johns block or with a Thomas-Hoover capillary melting apparatus and are uncorrected.

Isolation of Linustatin and Neolinustatin. Solvent-defatted flaxseed (*Linum usitatissimum* L., 500 g) was extracted with ethanol–water (1:1) at 70 °C. Fractionation of the solid material recovered by evaporating the aqueous ethanol solution (70 °C, ambient pressure) was monitored at each stage by bioassay with rat-feeding experiments described elsewhere.[2] Initially, this solid residue (72.4 g) was extracted with water; the resulting aqueous extract was similarly evaporated at 70 °C to yield 47.6 g of solid material which was, in turn, extracted with methanol. After evaporation of the combined methanolic extracts in vacuo, the residue (21.5 g) was dissolved in methanol; sufficient chloroform was added to provide a 2:1 solution of $CHCl_3$–MeOH. After removal of the resulting precipitate, the filtrate was evaporated in vacuo and yielded 7.5 g of product which was applied to a silicic acid column (180 g, Mallinckrodt) and eluted successively with 400 mL of $CHCl_3$, 800 mL of $CHCl_3$–MeOH (4:1), and 1400 mL of $CHCl_3$–MeOH (2:1). The selenium protective activity resided in the last of these three eluates; when evaporated to dryness in vacuo, this gave 3.4 g of product which was further fractionated by LC. A 2.5-g sample dissolved in 10 mL of methanol was injected into a 5.7 cm × 30 cm, C_{18}, μ-Bondapak column (Waters Associates). Elution was with methanol–water (15:85) at a rate of 250 mL/min. Three major peaks were eluted. The first LC peak, eluted at 3.9 min, was an inactive sugar which was not investigated further. The second LC peak (at 5.8 min, linustatin) and the third peak (at 9.7 min, neolinustatin) were active in the rat bioassay.[2] Peaks two and three also were examined by TLC [solvent system = $CHCl_3$–MeOH–17% NH_4OH (2:2:1)] and appeared to be homogeneous.

Linustatin (1a). Evaporation of the second LC peak in vacuo provided 0.85 g of **1a**, mp 123–123.5 °C, after recrystallization from ethanol: IR (KBr) 3450 (OH, very strong), 2240 (C≡N, weak) 1150 cm^{-1}; ORD $[\alpha]^{25}_D$ −37, $[\alpha]_{560}$ −41, $[\alpha]_{520}$ −48, $[\alpha]_{480}$ −59, $[\alpha]_{440}$ − 70, $[\alpha]_{400}$ −88, $[\alpha]_{360}$ −116, $[\alpha]_{320}$ − 163° (*c* 0.31, H_2O); ^{13}C NMR, in Table I; ^1H NMR (CD_3OD) δ 1.66, 1.67 (2 s, 6 H, methyl groups); (C_5D_5N) 1.64, 1.76 (2 s, 6 H, methyl groups).

Anal. Calcd for $C_{16}H_{27}NO_{11}$ (mol wt 409.39): C, 46.9; H, 6.7; N, 3.4. Found: C, 46.8; H, 7.0; N, 3.2.

Acetylation of 1a. Compound **1a** was acetylated by brief reflux in acetic anhydride–pyridine.[23] The resulting heptaacetate had a melting point of 183.5–184.5 °C (from ethanol): IR ($CHCl_3$) 2960, 2900, 1745 (carbonyl), 1370 (acetate), 1050, 905 cm^{-1}; ^1H NMR (C_6D_6) δ 1.22, 1.37 (2 s, 6 H, methyl groups of aglycon moiety), 1.82–1.87 (7 s, 21 H, acetate methyls).

Anal. Calcd for $C_{30}H_{41}NO_{18}$ (mol wt 703.65): C, 51.2; H, 5.9; N, 2.0. Found: C, 51.1; H, 6.0; N, 1.9.

Neolinustatin (1b). Evaporation of the third LC peak in vacuo provided 0.95 g of **1b**, mp 190–192 °C, after recrystallization from ethanol: IR (KBr) 3540 (OH, very strong), 2240 (C≡N, weak), 1150 cm^{-1}; ORD $[\alpha]^{25}_D$ −37, $[\alpha]_{560}$ −41, $[\alpha]_{520}$ −49, $[\alpha]_{480}$ −59, $[\alpha]_{440}$ −71, $[\alpha]_{400}$ −90, $[\alpha]_{360}$ −119, $[\alpha]_{320}$ −174° (*c* 0.37, H_2O); ^{13}C NMR, in Table I; ^1H NMR (C_5D_5N) δ 1.06 (t, 3 H, CH_3CH_2C, *J* = 8 Hz), 1.66 (s, 3 H, CH_3C), 2.01 (q, 2 H, CH_3CH_2C, *J* = 8 Hz).

Anal. Calcd for $C_{17}H_{29}NO_{11}$ (mol wt 423.42): C, 48.2; H, 6.9; N, 3.2. Found: C, 48.1; H, 7.2; N, 3.2.

Acetylation of 1b. Compound **1b** was acetylated by brief reflux in acetic anhydride–pyridine.[23] The resulting heptaacetate had a melting point of 171.5–173 °C (from ethanol): IR ($CHCl_3$) 2960, 2900, 1745 (ester carbonyl), 1370 (acetate), 1050 (br m), 905 cm^{-1}; ^1H NMR (C_6D_6) δ 0.88 (t, 3 H, CH_3CH_2C, *J* = 7 Hz), 1.65–1.88 (7 s, 21 H, acetate methyls), 4.15 (dd, 1 H on CH_2 at C-6, *J* = 12, 2 Hz), 4.26 (dd, 1 H on CH_2 at C-6, *J* = 12, 2 Hz), 4.44 (d, C-1β H, *J* = Hz), 4.68 (d, C-1α H, *J* = 7 Hz).

Anal. Calcd for $C_{31}H_{43}NO_{18}$ (mol wt 717.67): C, 51.9; H, 6.0; N, 2.0. Found: C, 51.7; H, 6.4; N, 1.9.

Enzymatic Hydrolysis of 1a. **1a** (100 mg) and 2.5 mg of β-glucosidase (EC No. 3.2.1.21, almond emulsin, Sigma Chemical Co.) were dissolved in 20 mL of water. The resulting solution

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(22) The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

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was incubated in a shaker bath 2 h at 37 °C. The reaction mixture then was evaporated to dryness in vacuo, and the products were fractionated by LC using a C_{18} μ-Bondapak column (6 mm × 30 cm); the eluting solvent was H_2O–MeOH (85:15), and the flow rate was 2.8 mL/min. Two reaction products were eluted: glucose (40.1 mg) at 3.8 min and a second peak (51.7 mg) at 6.0 min, identical with **2a**: mp 141–144 °C, after recrystallization from chloroform (lit.[15] mp 143–144 °C); ORD $[\alpha]^{25}_D$ −24, $[\alpha]_{560}$ −26, $[\alpha]_{520}$ −31, $[\alpha]_{480}$ −37, $[\alpha]_{440}$ −47, $[\alpha]_{400}$ −58, $[\alpha]_{360}$ −76, $[\alpha]_{320}$ −102° (*c* 1.03, H_2O) (lit.[15] $[\alpha]^{26}_D$ −27.5°); ^{13}C NMR, in Table I.

An authentic sample of **2a**, provided by Dr. David S. Seigler, University of Illinois at Urbana-Champaign, showed the following: ORD $[\alpha]^{25}_D$ −23, $[\alpha]_{560}$ −26, $[\alpha]_{520}$ −31, $[\alpha]_{480}$ −37, $[\alpha]_{440}$ −45, $[\alpha]_{400}$ −57, $[\alpha]_{360}$ −75, $[\alpha]_{320}$ −102, $[\alpha]_{290}$ −133° (*c* 1.43, H_2O); ^{13}C NMR, in Table I.

Enzymatic Hydrolysis of 1b. **1b** (100 mg) was treated with β-glucosidase as described for **1a**. The reaction product was fractionated by LC similarly, and two reaction products were eluted: glucose (41.8 mg) at 3.8 min and a second peak, identical with **2b** (56.6 mg), at 9.4 min: mp 120–122 °C, after crystallization from water (lit.[15] mp 123.5–124.5 °C); ORD $[\alpha]^{25}_D$ −18, $[\alpha]_{560}$ −20, $[\alpha]_{520}$ −23, $[\alpha]_{480}$ −28, $[\alpha]_{440}$ −34, $[\alpha]_{400}$ −42, $[\alpha]_{360}$ −54, $[\alpha]_{320}$ −72° (*c* 0.80, H_2O) (lit.[15] $[\alpha]^{25}_D$ −19°); ^{13}C NMR, in Table I.

Authentic **2b** was obtained from a mixture of **2a** and **2b** provided by Dr. R. C. Clapp, U.S. Army Natick Development Center, and isolated originally from *Lotus australis*; 82 mg was separated by LC. Samples were injected into a 7.8 mm × 30 cm C_{18} μ-Bondapak column and eluted at 2 mL/min with H_2O–MeOH (80:20); peaks were eluted at 6.8 min (unidentified, 1.3 mg), 11.2 min (unidentified, 1.5 mg), 14.0 min (**2a**, 22.5 mg), and 18.0 min (**2b**, 55.4 mg): mp 122.5–123.0 °C, after crystallization from water; ORD $[\alpha]^{25}_D$ −16, $[\alpha]_{560}$ −18, $[\alpha]_{520}$ −23, $[\alpha]_{480}$ −28, $[\alpha]_{440}$ −34, $[\alpha]_{400}$ −42, $[\alpha]_{360}$ −53, $[\alpha]_{320}$ −73, $[\alpha]_{280}$ −104, $[\alpha]_{240}$ −160° (*c* 0.62, H_2O); ^{13}C NMR, in Table I.

Amygdalin (1d). An authentic sample of **1d** provided by Dr. David S. Seigler showed the following: ORD $[\alpha]^{25}_D$ −40, $[\alpha]_{560}$ −45, $[\alpha]_{520}$ −53, $[\alpha]_{480}$ −64, $[\alpha]_{440}$ −79, $[\alpha]_{400}$ −98, $[\alpha]_{360}$ −130, $[\alpha]_{320}$ −178, $[\alpha]_{280}$ −261° (*c* 0.33, H_2O) (lit.[9] $[\alpha]^{20}_D$ −41.9°); ^{13}C NMR, in Table I.

Gentiobiose (1c). β-Gentiobiose from Sigma Chemical Co. was used for determination of the ^{13}C NMR spectrum; see Table I.

Complete Acid Hydrolysis of 1b with Mineral Acid. A 0.012-g portion of **1b** was refluxed 3 h with 10 mL of 5% aqueous HCl. The mixture then was evaporated to dryness in vacuo and was analyzed by TLC [solvent system = 1-butanol–acetone–H_2O (4:5:1)]. The only monosaccharide detected was glucose.

Partial Hydrolysis of 1b with Mineral Acid. A 0.020-g portion of **1b** was dissolved in 10 mL of 0.5 N HCl at ambient temperature. After 1 h, the solution was evaporated to dryness in vacuo and was analyzed by LC. Sample was injected into a Waters 3.9 mm × 30 cm carbohydrate μ-Bondapak column and was eluted with MeCN–H_2O (77:23) at 2 mL/min. The following components were recorded [elution time (identity)]: 2.1 min (**2b**), 2.8 min (unidentified), 3.3 min (**1b**), 4.7 min (**2c**), 5.4 min (unidentified), 6.7 min (unidentified), 9.7 min (**1c**). Components were identified by comparison of their retention times with those of authentic reference compounds injected individually.

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Registry No. **1a**, 72229-40-4; **1a** heptaacetate, 72229-41-5; **1b**, 72229-42-6; **1b** heptaacetate, 72229-43-7; **1c**, 5996-00-9; **1d**, 29883-15-6; **2a**, 554-35-8; **2b**, 534-67-8; **2c**, 492-61-5.

The C-13 Configuration of the Bromine-Containing Diterpene Isoaplysin-20. Synthesis of Debromoisoaplysin-20 and Its C-13 Epimer

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Syntheses of the tricyclic diterpenes 13-epidebromoisoaplysin-20 (**14**) and debromoisoaplysin-20 (**15**) from methyl copalate (**3**) are described. Methyl isocopalate (**4**) prepared from **3** was converted into the corresponding alcohol **8**. By epoxidation of **8** a 1:1 mixture of isomeric epoxides was obtained; the reductive opening of the α-epoxide (**13**) afforded **14**. Hydroxylation of **4** gave the diol ester **16**, which on oxidation with dimethyl sulfoxide–acetic anhydride yielded **18a**. Transformation of **18a** into the ethylene thioketals **20** and **20a**, followed by desulfuration of the mixture to the hydroxy ester **21**, and subsequent lithium aluminium hydride reduction afforded **15**. The stereochemical features of the epoxides **9** and **13** and the diol ester **16** as well as those at C-13 of **14** and **15** were determined by analysis of their ^{13}C NMR spectra. On the basis of the comparison of the ^1H NMR signals of the methyl groups of **14** and **15** and the ones of the acetoxymethylene groups of their monoacetates **14a** and **15a** with those reported for the methyls of the bromine-containing diterpene isoaplysin-20 (**1**) and the acetoxymethylene of its monoacetate (**1a**), the stereochemistry of the natural product was established.

Recently Yamamura and Terada isolated from the sea hare *Aplysia kurodai* a small amount of a bromine-containing tricyclic diterpene, named isoaplysin-20 (**1**). The structure elucidation of **1** was based on the comparison of spectroscopic data with the related bicyclic diterpene aplysin-20 (**2**), also isolated from the same source, and on biogenetic considerations; the configuration at C-13, however, remained undetermined.[1,2] Since the configuration of that type of tertiary alcohol could be, on the basis of previous experience,[3] easily determined by ^{13}C NMR spectroscopy, the synthesis of debromoisoaplysin-20 and/or its C-13 epimer was planned in order to obtain enough material for carrying out these determinations, in the hope that by comparison of the available ^1H NMR data of **1** with those of the synthetic product(s) the complete stereochemistry of isoaplysin-20 could be established.

With the methyl ester of the readily available copalic acid (**3**)[4,5] as starting material and under the conditions

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