mp 102–104 °C; $[\alpha]_D$ +5.4 (MeOH)]; TLC (benzene/acetone 3:1) R_f 0.40. Anal. Calcd for $C_{15}H_{21}NO_2$: C, 61.0; H, 7.2; N, 4.7. Found: C, 61.1; H, 7.3; N, 4.9.

***tert*-(Butyloxycarbonyl)-D-tyrosine Methyl Ester.** This was obtained by the same procedure from D-TyrOMe·HCl (50 g, 0.216 mol), K_2CO_3 (29.9 g, 0.216 mol), *t*-BuOH (100 mL), and di-*tert*-butyl dicarbonate (45.8 g, 0.210 mol): yield 50.3 g (79%); mp 102–109 °C; $[\alpha]^{25}_D$ −10.50° (*c* 2, MeOH). Anal. Calcd for $C_{15}H_{21}NO_2$: C, 61.0; H, 7.2; H, 4.7. Found: C, 61.2; H, 7.3; N, 4.8.

Boc-*O*-isopropyltyrosine (Procedure A, from Boc-L-TyrOMe). A mixture of Boc-L-TyrOMe (5.9 g, 20 mmol), K_2CO_3 (3.0 g, 22 mmol), 18-crown-6 (0.6 g, 2.2 mmol), and isopropyl methanesulfonate (3.0 g, 22 mmol) in benzene (90 mL) and DMF (10 mL) was stirred and heated to boiling in an 0.5-L flask equipped with an azeotropic distilling receiver and a condenser. After 5 h the reaction mixture was cooled and cold water (50 mL) added. The organic layer was separated, washed with water (twice), dried with $MgSO_4$ and evaporated. The residual colorless oil was dissolved in methanol (20 mL), cooled in an ice bath, and mixed with a solution of NaOH (0.9 g, 22.5 mmol) in water (10 mL) and left to stand for 0.5 h. After removal of methanol at room temperature, the residue was diluted with an ice–water mixture (100 mL) and acidified with 2 M HCl to pH 2. The precipitate was extracted with AcOEt (100 and 50 mL). The combined organic solutions were washed with NaCl solution (twice) and dried with $MgSO_4$. After removal of ethyl acetate, the crystalline residue was crystallized from *n*-hexane: yield 3.9 (61%); mp 87–91 °C; $[\alpha]^{25}_D$ +24.6° (*c* 1, EtOH).

Boc-*O*-isopropyl-L-tyrosine (Procedure B, from Boc-L-Tyr). A mixture of Boc-L-Tyr (5.6 g, 20 mmol), K_2CO_3 (6.0 g, 44 mmol), 18-crown-6 (0.6 g, 2.2 mmol), and isopropyl methanesulfonate (6.0 g, 44 mmol) in benzene (90 mL) and DMF (10 mL) was stirred and heated to boiling for 6 h as in procedure A. Further workup was similar to procedure A, except that the basic hydrolysis was prolonged to 1 h: yield 3.5 g (55%); mp 86–90 °C; $[\alpha]^{26}_D$ +23.7° (*c* 1, EtOH).

Registry No. Boc-Tyr-OMe, 4326-36-7; Boc-Tyr, 3978-80-1; Boc-D-Tyr-OMe, 76757-90-9; z-Tyr, 1164-16-5; CHO-Tyr, 13200-86-7; Boc-Tyr(Me), 53267-93-9; Boc-D-Tyr(Me), 68856-96-2; Boc-Tyr(Et), 76757-91-0; Boc-D-Tyr(Et), 76757-92-1; Boc-Tyr(*n*-Pr), 76757-93-2; Boc-Tyr(*i*-Pr), 76757-94-3; z-Tyr(Et), 66147-90-8; CHO-Tyr(Me), 76757-95-4; L-TyrOMe·HCl, 3417-91-2; *n*-propyl methanesulfonate, 1912-31-8; isopropyl methanesulfonate, 926-06-7; D-tyrosine methyl ester HCl, 3728-20-9; D-tyrosine, 556-02-5.

3′-*O*-Methylevomonoside: A New Cytotoxic Cardiac Glycoside from *Thevetia ahouia* A. DC (Apocynaceae)

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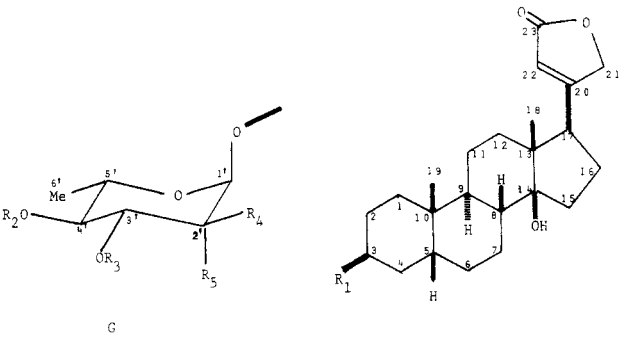
During the course of our search for plants having tumor inhibitory constituents, an ether extract of the title plant yielded a crystalline compound which exhibited strong cytotoxic activity against the human epidermoid carcinoma of the nasopharynx (KB) test system.[1] We present evidence that it is the previously unknown 3-*O*-methyl ether (3) of evomonoside (2)[2] and is thus the C-2′ epimer of neriifolin (4).[3]

Table I. ^{1}H NMR Chemical Shifts (δ) and Coupling Constants (Hertz, in Parentheses) for 3′-*O*-Methylevomonoside (3) and Neriifolin (4)

	3	4
3	3.97 m	3.97 m
16 α,β	2.0–2.25 m	2.0–2.25 m
17	2.28 dd (9.0, 5.3)	2.78 dd (8.8, 5.0)
18	0.88 s	0.88 s
19	0.94 s	0.97 s
21 α,β	4.82 dd (18.0, 1.5)	4.82 dd (18.0, 1.5)
	4.99 dd (18.0, 1.5)	4.98 dd (18.0, 1.5)
22	5.88 t (1.5)	5.88 t (1.5)
1′	4.92 d (1.7)	4.86 d (4.4)
2′	4.02 dd (3.1, 1.7)	3.58 dd (8.9, 4.4)
3′	3.42 dd (9.2, 3.1)	3.25 t (8.9)
4′	3.50 t (9.1)	3.15 t (9.0)
5′	3.73 dq (9.0, 6.3)	3.74 dq (9.0, 6.3)
6′	1.29 d (6.3)	1.26 d (6.3)
MeO	3.50 s	3.69 s



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	R_1	R_2	R_3	R_4	R_5
1	OH	-	-	-	-
2	G	H	H	H	OH
3	G	H	Me	H	OH
3a	G	Ac	Me	H	OAc
4	G	H	Me	OH	H

3′-*O*-Methylevomonoside (3), mp 203–204 °C, $[\alpha]^{25}_D$ −20.6°, and neriifolin (4) appeared on TLC with nearly identical R_f values. 3 displayed a hardly discernible molecular ion peak at *m/e* 534 (EI mass spectrum shifted to *m/e* 750 ($M^{+\cdot}$) in its Me_3Si derivative), which, combined with its elemental analysis, led to molecular formula $C_{30}H_{46}O_8$. The IR (KBr) spectrum of 3, which displayed characteristic dienone (1785, 1740 cm^{-1}) and broad hydroxyl (3490, 3420 cm^{-1}) bands, was very similar to that of neriifolin (4). The mass spectrum of 3 exhibited readily interpretable fragmentation peaks, *m/e* 357 [M − (sugar − H)], 339 (357 − H_2O), 246 (357 − $C_6H_7O_2$), 203 [246 − (CH_3 + CO)], 161 [M − (aglycon − H)], and 74 (base, $[CH_3OCH{=}CHOH]^{+\cdot}$), all suggesting 3 to have digitoxigenin linked to a deoxy-*O*-methyl hexose moiety.

The ^{1}H (Table I) and ^{13}C (Table II) NMR spectra of 3 and neriifolin (4) were very similar except for the sugar absorptions, strongly indicating that 3, like 4, is a glycoside of digitoxigenin (1). The ^{13}C NMR shifts of neriifolin (4)

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(2) Zorbach, W. W.; Valiaveedan, G. D.; Kashelikar, D. V. *J. Org. Chem.* **1962**, *27*, 1766.

(3) Helpenberger, H.; Reichstein, T. *Helv. Chim. Acta* **1948**, *31*, 1470. The sample of neriifolin used in this investigation was isolated in this laboratory from *Thevetia peruviana* (Apocynaceae). Its identity was established by direct comparison with an authentic sample.

0022-3263/81/1946-1946$01.25/0

Table II. ^{13}C NMR Chemical Shifts (δ) for 3′-*O*-Methylevomonside (3), Neriifolin (4), and Digitoxigenin (1)

	3	4	1
1	30.4 t[a]	30.6[a]	30.0
2	26.5 t[a]	26.5[a]	28.0
3	71.7 d	73.3	66.8
4	29.4 t[a]	30.0[a]	33.5
5	36.5 d	36.9	35.9[a]
6	26.6 t[a]	26.5[a]	27.1
7	21.2 t[b]	21.2[b]	21.6[b]
8	41.8 d	41.8	41.9
9	35.7 d	35.9	35.8[a]
10	35.2 s	35.3	35.8
11	21.4 t[b]	21.4[b]	21.7[b]
12	40.0 t	40.0	40.4
13	50.3 s	50.3	50.3
14	85.5 s	85.5	85.6
15	33.1 t	33.2	33.0
16	26.9 t	26.9	27.3
17	50.9 d	50.9	51.5
18	15.8 q	15.8	16.1
19	23.8 q	23.9	23.9
20	174.6 s	174.6	177.1[c]
21	73.5 t	73.4	74.5
22	117.7 d	117.8	117.4
23	174.6 s	174.6	176.3[c]
1′	97.3 d	97.2	
2′	67.4 d	73.0	
3′	81.4 d	84.7	
4′	71.7 d	74.7	
5′	67.7 d	67.5	
6′	17.6 q	17.5	
MeO	57.0 q	60.6	

[a-c] Values in any vertical column may be reversed.

had not been reported previously; they are assigned in accordance with the assignments made for digitoxigenin (1) itself.[4] The only significant differences in ^{13}C NMR shifts among the three compounds came in the vicinity of C-3 where they possess different substituents. That 3 possesses the same β configuration at C-3 as does 4 is clear from the similarity in appearance of their C-3 proton absorptions: both give broad multiplets about 20 Hz wide.

From the vicinal ^1H–^1H coupling constants in the sugar portion of 3 (Table I), it is clearly a mannose derivative; H-3′, H-4′, and H-5′ are axial from the large values of $J_{3',4'}$ (9.2 Hz) and $J_{4',5'}$ (9.0 Hz), and H-2′ is equatorial from the small value of $J_{2',3'}$ (3.1 Hz). The 1.7 Hz coupling constant observed for $J_{1',2'}$ further suggests an α configuration ($J_{1,2}$ = 1.8-1.9 in α-mannosides, $J_{1,2}$ = 1.1 Hz in β-mannosides[5]); the $J_{1',2'}$ (4.4 Hz) observed for neriifolin (4) confirms its α configuration. The coupling pattern for H-5′ and the C-6′ methyl doublet indicates a rhamnose (6-deoxymannose) derivative. The downfield location of the C-3′ absorption clearly shows the presence of the methoxyl at C-3′, as in neriifolin (4); the sugar is thus 3-*O*-methylrhamnose, previously obtained in the L form from the hydrolysis of black spruce wood.[6]

That the new cardenolide, like all previous cardenolides containing a rhamnose grouping, is an L-rhamnose derivative is indicated by its molecular rotation of −110°; D-rhamnosides have very large positive molecular rotations, especially if α.[2] The reasonableness of the stereochemical assignments in structure 3 can be checked by comparing the molecular rotation of 3 with those of the closely related substances evomonoside (2, $[M]_D$ −159°)[2] and neriifolin (4, $[M]_D$ −267°):[3] the change of +49° from 2 to 3 roughly parallels the change of +75° in going from α-L-rhamnose ($[M]_D$ −14°)[7] to 3-*O*-methyl-α-L-rhamnose ($[M]_D$ 62°), and the change of +157° from 4 to 3 is similar to the change of +179° in going from α-L-thevetose ($[M]_D$ −117°)[8] to its C-2′ epimer 3-*O*-methyl-α-L-rhamnose.

Acetylation of 3 gave diacetate **3a**, showing a tertiary hydroxyl in the IR, with a fragmentation pattern showing expected peak shifts for a diacetylated sugar [e.g., *m/e* 161 in 3 shifted to *m/e* 245 (base) in **3a**].

Experimental Section

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Carbon and hydrogen analyses were carried out by University Analytical Center, Tucson, AZ. Optical rotations were measured, by using a Perkin-Elmer 241 MC polarimeter. Infrared (IR) spectra were run on a Beckman IR-33 spectrometer. ^1H NMR spectra were run at 60 MHz on a Varian EM 360L spectrometer or at 250 MHz on a Bruker Spectrospin spectrometer, and ^{13}C NMR spectra were run at 22.63 MHz, using a Bruker WH-90 spectrometer; in both cases shifts are given as parts per million downfield from Me_4Si (δ). Proton and carbon shift assignments in the sugar portions of 3 and 4 were verified by ^1H–^1H and ^1H–^{13}C decoupling. Mass spectra were recorded on a Varian MAT 311A spectrometer (intensities of the ions are given in parentheses).

3′-*O*-Methylevomonoside (3). The ground whole plant of *Thevetia ahouai* (2500 g), collected in Mexico (May 1972), was exhaustively extracted with ether in a Soxhlet extractor and the resulting vacuum-dried ether extract (150 g) was stirred magnetically with *n*-hexane for 4 h, left overnight, and filtered and the residue washed with *n*-hexane. The combined hexane filtrate, after removal of the solvent under vacuum, was stirred magnetically with methylene chloride for 6 h and filtered, the solvent was removed under vacuum, and the resulting residue was subjected to column chromatography (EM SiO_2-60, 1200 g), using ethyl acetate as the eluent. Fractions judged by TLC to contain 3 were combined, and the solvent was removed under vacuum; preparative TLC (EM SiO_2-60 PF-254) followed by crystallization from aqueous MeOH gave 3 as tiny colorless needles: mp 203–204 °C; $[\alpha]^{25}_D$−20.6° (MeOH).

The IR [($CHCl_3$) 3600, 3460, 3005, 1788, 1742, 1620, 1442, 1376, 1096, 1040, 975, 915, 890 and 788 (KBr) cm^{-1}] and mass [*m/e* 534 (M^+·, 0.2), 403 (9.8), 385 (2), 375 (10.5), 357 (89.4), 356 (48.4), 339 (96.4), 246 (56.8), 231 (11.3), 203 (96), 181 (22.9), 177 (22.8), 161 (30.5), 149 (16.3), 147 (22.5), 135 (18), 129 (24), 121 (25.8), 111 (25.3), 109 (30.4), 107 (32.5), 105 (25.4), 95 (48), 85 (52.9), 74 (100), 67 (37), 55 (34)] spectra were in accord with structure 3.

Anal. Calcd for $C_{30}H_{46}O_8$: C, 67.41; H, 8.61. Found: C, 67.45; H, 9.09.

3′-*O*-Methylevomonoside (3) demonstrated an activity of 2.7 × 10^{-3} μg/mL. Activity in the KB test system is defined as ED_{50} ≤ 20 μg/mL.

2′,4′-Di-*O*-acetyl-3′-*O*-methylevomonoside (3a), prepared from Ac_2O–pyridine (25 °C, 24 h), mp 113–115 °C, had IR [(KBr) 3500, 1780, 1735 (br), 1620, 1225 cm^{-1}] and mass [*m/e* 618 (M^+·, not observed), 357 (57), 339 (49), 246 (22), 245 (100), 231 (7), 213 (24), 203 (39), 153 (63), 116 (27), 111 (43), 95 (31), 85 (28), 74 (26), 44 (64), 43 (84)] spectra in accord with structure **3a**.

Anal. Calcd for $C_{34}H_{50}O_{10}$: C, 66.02; H, 8.09. Found: C, 66.00; H, 8.36.

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Registry No. 1, 143-62-4; 3, 4356-33-6; **3a**, 76756-40-6; 4, 466-07-9.

(4) Tori, K.; Ishii, H.; Wolkowski, Z. W.; Chachaty, C.; Sangave, M.; Piriov, F.; Lukacs, G. *Tetrahedron Lett.* **1973**, 1077.
(5) Actona, C.; Haasnoot, C. A. G. *Org. Mag. Reson.* **1980**, *13*, 417.
(6) Gorrod, A. R. N.; Jones, J. K. N. *J. Chem. Soc.* **1954**, 2522. It is not clear whether the optical rotation given is for the α isomer (the form which usually crystallizes) or for a mixture of α and β; we have assumed the former. If the latter is the case, the molecular rotation would be up to 35° less and the calculated moelcular rotation for 3 would be even closer to the observed value.
(7) Windholz, M. "Merck Index", 9th ed., 1976; p 1060.
(8) Reference 7, p 1197.