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NEW MACROCYCLIC TRICHOTHECENES FROM *BACCHARIS MEGAPOTAMICA*

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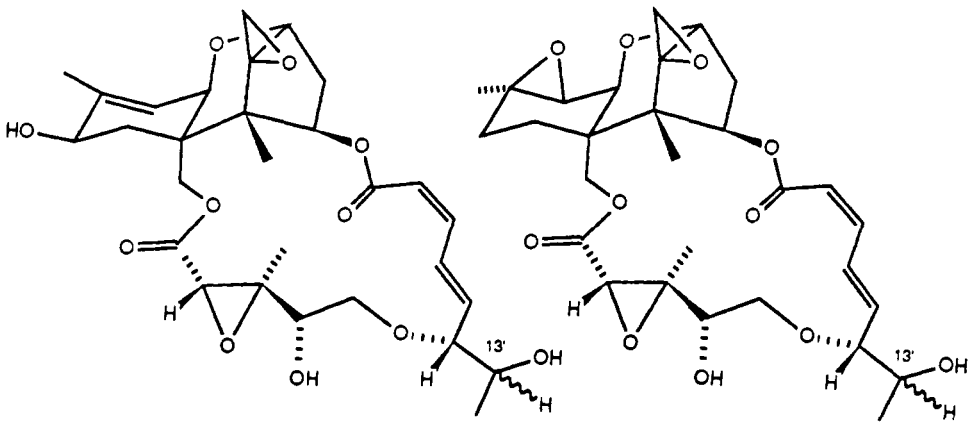
ABSTRACT.—The isolation and structural elucidation of biologically active baccharinoids B1 [**11a**], B2 [**12a**], B3 [**5a**], and B7 [**6a**] are reported with crystal structure determinations of baccharinoid B7 and of the triacetate of baccharinoid B2. All four compounds are isomeric with **11a**/**12a** and **5a**/**6a** being epimeric at C13′.

During the course of a program directed toward obtaining anticancer agents from plants, we reported the isolation of a set of macrocyclic trichothecenes, called baccharinoids, from the Brazilian shrub, *Baccharis megapotamica* Spreng. (Asteraceae) (1). Subsequent work with this plant extract revealed that, in addition to the four original baccharinoids (B4 [**1**], B5 [**2**], B6 [**3**], and B8 [**4**]) reported, this plant contained a further four major baccharinoids (B1, B2, B3, and B7) and numerous minor baccharinoids (2). A large scale collection of *B. megapotamica* has been made from which appreciable quantities of the baccharinoids have been isolated, including a number of new baccharinoids (3). In this report we give the details of the isolation and characterization, including the absolute stereochemistries, of the remaining major baccharinoids, B1, B2, B3, and B7.

RESULTS AND DISCUSSION

The crude extract from 1800 kg of plant material was subjected to a series of solvent



1 C13′=*R*
3 C13′=*S*

2 C13′=*R*
4 C13′=*S*

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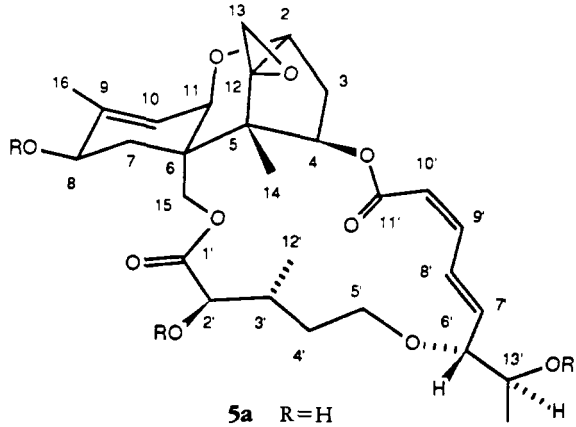
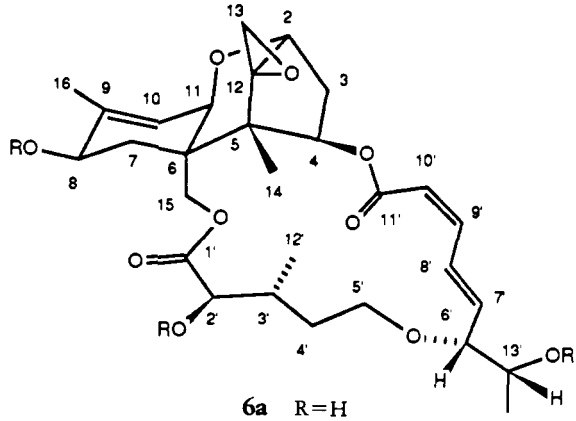
partitions and a ferric gel precipitation to yield 4.7 kg of resin. This material was subjected to a large scale chromatography over Si gel to yield ten fractions (I-X) (3). Fraction VIII (162 g) was taken up in CH_2Cl_2, washed with 2.5% aqueous NaOH, and the CH_2Cl_2 soluble material subjected to a series of chromatographies (see Experimental section) to give two new macrocyclic trichothecenes (baccharinoids B3 [**5a**] and B7 [**6a**]) whose molecular formulas are $C_{29}H_{40}O_{10}$ as shown by hrms. Upon methanolysis, both **5a** and **6a** gave 8β-hydroxyverrucarol [**7**] (1), and upon acetylation (Ac_2O/pyridine), **5a** and **6a** gave triacetates **5b** and **6b**, respectively.

Comparison of the spectral data (^1H nmr, ir, uv, and ms) for **5a** and **6a** with 8β-hydroxyroridin A [**8**] (4) suggested that 8β-hydroxyroridin A [**8**], **5a** and **6a** are dias-

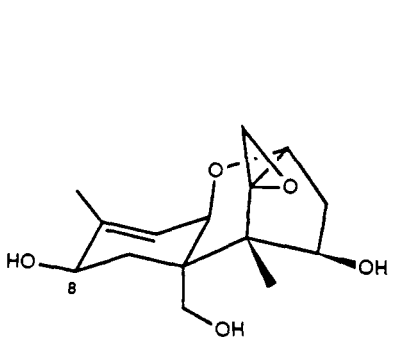


5a R=H
5b R=Ac

tereomers of one another. Furthermore, previous baccharinoids (1,2) have been isolated as sets of diastereomers, epimeric at C13′, suggesting that **5a** and **6a** might have the same relationship. Selective hydrogenation of **5a** and **6a** gave the 7′,8′,9′,10′-tetrahydro derivatives **9a** and **9b**, respectively, which upon oxidation (pyridinium chlorochromate) gave the same diketone **10**. Thus, triols **5a** and **6a** are epimeric at C13′. To determine the stereochemistry at the remaining centers in question (C2′, C3′, and C6′), a single-crystal X-ray diffraction analysis of baccharinoid B7 [**6a**] was performed. These results established the stereochemistries as shown in structures **5a**



6a R=H
6b R=Ac



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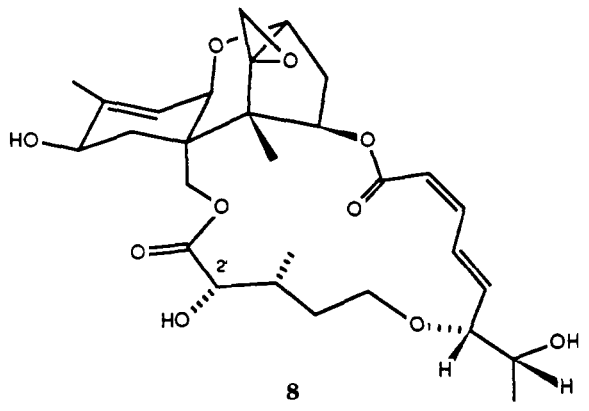

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and **6a** and illustrated in Figure 1. Interestingly, baccharinoid B7 [**6a**] is epimeric with 8β-hydroxyroridin A [**8**] at C2′, and experiments have shown that *B. megapotamica* in vivo transforms **8** to **6a** (5).

Fraction IX (1.16 kg) was subjected to a series of further chromatographies to yield 84 g of a solid material containing baccharinoids B1 [**11a**], B2 [**12a**], and B4 [**1**] (3). A small portion of this material was subjected to hplc to give pure **11a** and **12a** which were shown by hrms to have molecular formulas of $C_{29}H_{40}O_{10}$, isomeric with baccharinoids B3 and B7. Hydrolysis of **11a** and **12a** gave 8β-hydroxyverrucarol [**7**], and acetylation of **11a** and **12a** gave triacetates **11b** and **12b**, respectively. ^{1}H- and ^{13}C-nmr data suggested that baccharinoids B1 [**11a**] and B2 [**12a**] are epimeric; however, these same data also indicated that **11a** and **12a** are positional isomers of baccharinoids B3 and B7. Selective hydrogenation of the dienic systems in **11a** and **12a** gave the corresponding tetrahydro derivatives **13a** and **13b**, respectively. Oxidation (pyridinium

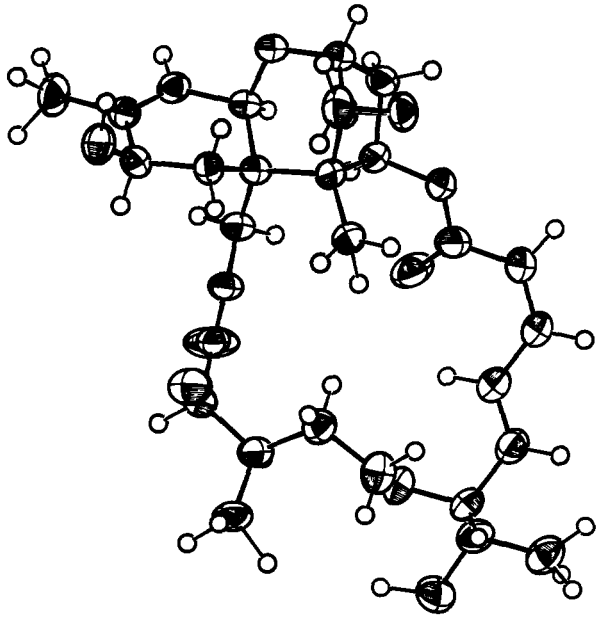
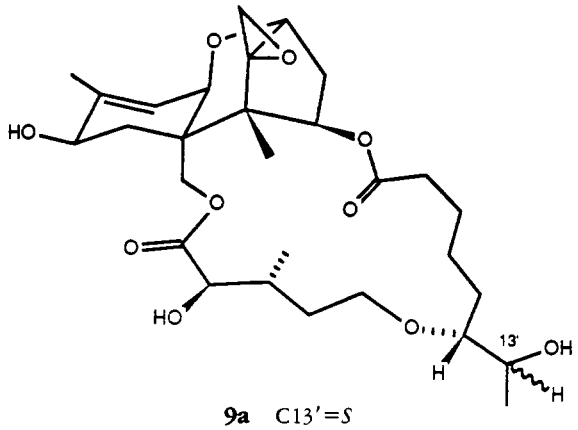
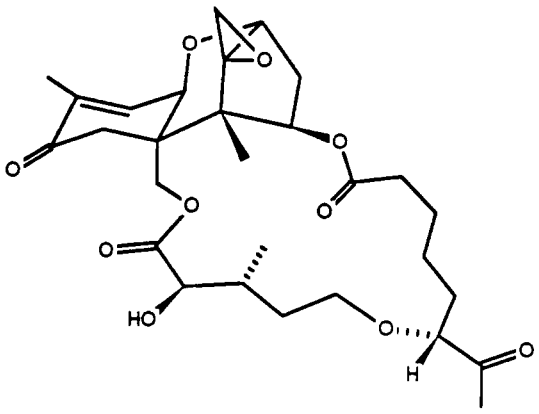


FIGURE 1. ORTEP drawing of **6a**. Thermal ellipsoids for the C and O atoms are drawn to enclose the 50% probability level, and the H atoms are shown as spheres of arbitrary radius. The H atom of the O2′ hydroxyl group was not located and is not shown.



9a C13′=*S*
9b C13′=*R*



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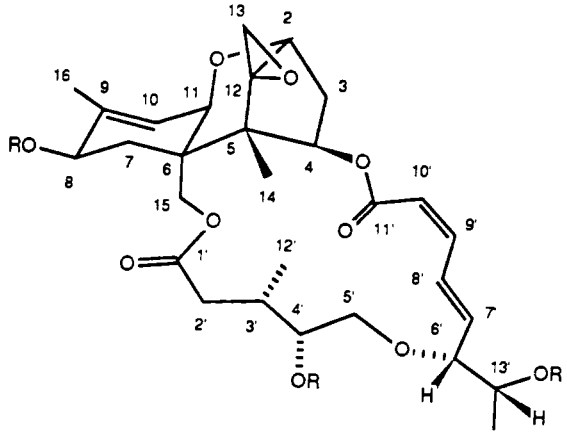
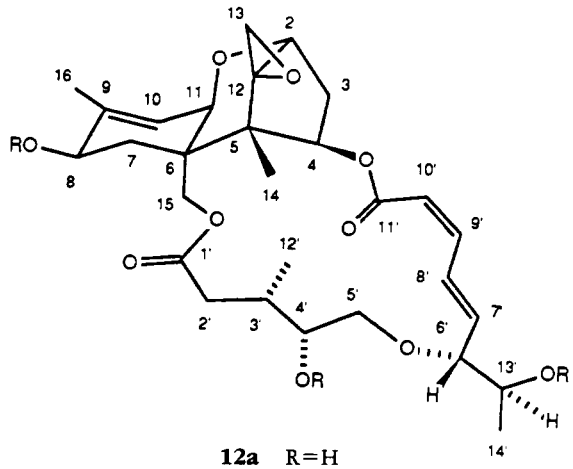
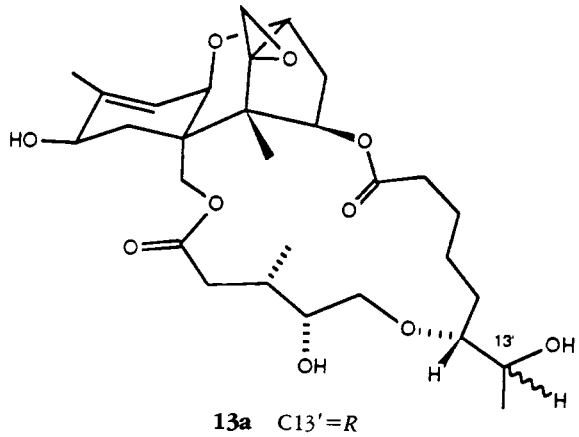


11a R=H
11b R=Ac

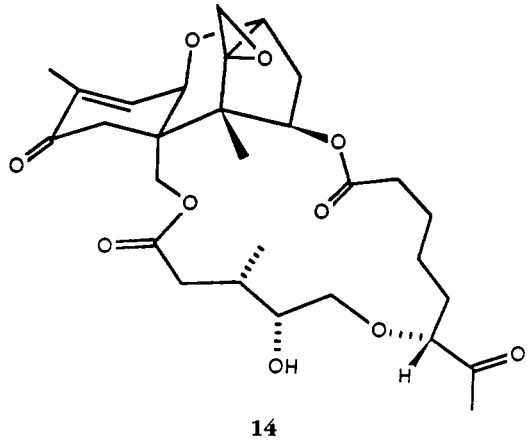
chlorochromate) of both **13a** and **13b** gave the same diketone **14**, establishing that **11a** and **12a** are epimeric at C13′. Neither baccharinoid B1 [**11a**] nor baccharinoid B2 [**12a**] gave crystals suitable for X-ray diffraction analysis; however, baccharinoid B2 triacetate [**12b**] did give suitable crystals. A single-crystal X-ray diffraction analysis of



12a R=H
12b R=Ac



13a C13′=*R*
13b C13′=*S*



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12b established the relevant stereochemistries as shown in structures **11** and **12** and illustrated in Figure 2.

Evidence has accumulated to support the hypothesis that *B. megapotamica* (5,6) and a related Brazilian plant, *Baccharis coridifolia* (7) acquire roridins from a fungal source. In the case of *B. megapotamica*, the roridins are metabolically A-ring oxygenated to the

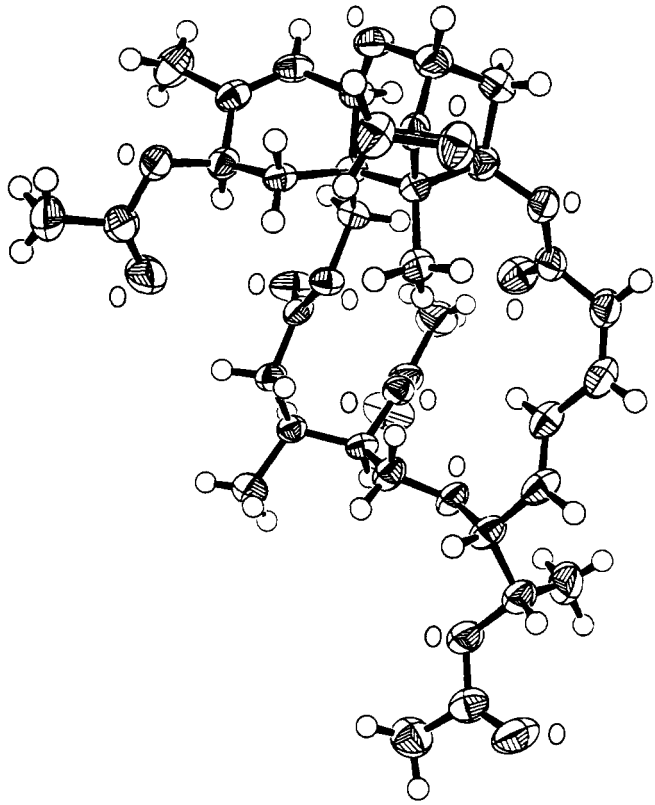

FIGURE 2. ORTEP drawing of **12b**. The C and O atoms are depicted as 50% ellipsoids; the H atoms are shown as 0.1Å spheres. The oxygen atoms have been labeled.

baccharinoids (5). The baccharinoids are typically present in the plants as sets of diastereomers epimeric at C13′. All of the roridins (roridins A, D, and E) and baccharinoids in this plant have *R* configurations at C6′ (1,3). The major epimers (e.g., baccharinoids B1, B4, B5, and B7) are *threo* (C6′*R*, C13′*R* and the minor epimers (e.g., baccharinoids B2, B3, B6, and B8) are *erythro* (C6′*R*, C13′*S*). The *erythro* and *threo* series can be distinguished by nmr spectroscopy (3). In the ^{1}H-nmr spectra of *erythro* acetates (e.g., baccharinoids B2 triacetate [**12b**] and B3 triacetate [**5b**]), the C13′ protons exhibit an eight line pattern (dd, $J_{6',13'} \approx 3.5$ Hz and $J_{13',14'} \approx 6.5$ Hz), whereas for the *threo* series (e.g., baccharinoids B1 triacetate [**11b**] and B7 triacetate [**6b**]), H13′ appears as a five-line multiplet (dd, $J_{13',14'} \approx J_{6',13'} \approx 6.5$ Hz). In addition, the chemical shifts for H13′ in the acetates are at consistently higher frequencies for the *threo* series (C13′*R*, as in **11b** and **6b**]) than for those observed in the *erythro* series (C13′*S*, as in **12b** and **5b**). The other consistent pattern observed is in the ^{13}C spectra. The chemical shifts of C13′ in the *threo* series (e.g., baccharinoids B1 [**11a**] and B7 [**6a**]) are at ca. 1-2 ppm higher frequency relative to the C13′s in the *erythro* series (e.g., baccharinoids B2 [**12a**] and B3 [**5a**]). Furthermore, C6′ resonates at a consistently higher frequency

TABLE 1. ^{1}H-nmr Spectral Data for Raccharinoids B1 [**11a**], B2 [**12a**], B3 [**5a**], B7 [**6a**], and Their Acetates

Position	Compounds							
	B1 [**11a**]	B1 Triacetate [**11b**]	B2 [**12b**]	B2 Triacetate [**12b**]	B3 [**5a**]	B3 Triacetate [**5b**]	B7 [**6a**]	B7 Triacetate [**6b**]
2	3.86 d (4.5)	3.86 d (4.9)	3.87 d (5.0)	3.87 d (5.0)	3.87 m	3.87 d (4.9)	3.87 d (4.0)	3.87 d (4.0)
3α	2.44 m	2.45 dd (8.0, 15.2)	2.45 m	2.45 dd (8.0, 15.0)	2.44 dd (8.4, 15.3)	2.45 dd (8.1, 15.4)	2.44 dd (8.1, 15.2)	2.45 dd (8.2, 15.3)
3α	2.20 m	2.20 m	2.20 m	2.20 m	2.20 m	2.19 m	2.22 m	2.20 m
4	5.77 m	5.78 dd (3.8, 7.7)	5.77 m	5.80 dd (3.6, 8.0)	5.75 dd (4.5, 8.4)	5.75 m	5.76 dd (4.7, 8.1)	5.75 dd (4.7, 8.1)
7	2.20 m	2.15 m	2.20 m	2.20 m	2.10 m	ca. 2.00 m	2.10 m	ca. 2.00 m
8	4.01 m	5.21 m	3.85 m	5.22 m	4.01 dd (5.3, 10.3)	5.11 dd (6.3, 9.9)	4.01 dd (4.9, 9.4)	5.11 dd (6.1, 9.6)
10	5.50 d (5.3)	5.57 d (5.3)	5.49 d (5.3)	5.58 d (5.3)	5.49 d (5.2)	5.57 d (5.4)	5.50 d (5.4)	5.57 d (5.5)
11	3.63 d (5.3)	3.52 d (5.3)	3.64 d (5.3)	3.62 d (5.3)	3.62 m	3.63 d (5.4)	3.59 m	3.63 d (5.5)
13	2.85, 3.13 AB (4.0)	2.81, 3.13 AB (4.0)	2.85, 3.13 AB (4.0)	2.82, 3.13 AB (4.0)	2.85, 3.13 AB (4.0)	2.83, 3.14 AB (4.0)	2.85, 3.13 AB (4.0)	2.83, 3.14 AB (4.0)
14	0.77 s	0.78 s	0.77 s	0.78 s	0.79 s	0.77 s	0.78 s	0.77 s
15	3.76, 4.68 AB (12.4)	4.08, 4.58 AB (12.3)	4.00, 4.66 AB (12.4)	4.06, 4.62 AB (13.0)	3.91, 4.85 AB (12.4)	3.93, 4.74 AB (12.5)	3.91, 4.89 AB (12.3)	3.93, 4.76 AB (12.6)
16	1.83 s	1.71 s	1.83 s	1.71 s	1.83 s	1.71 s	1.83 s	1.71 s
2′	2.20 m	2.25 m	2.25 m	2.20 m	4.16 d (4.2)	5.03 d (5.6)	4.18 d (4.0)	5.05 d (5.3)
3′	2.43 m	2.67 m	2.40 m	2.68 m	2.28 m	ca. 2.10 m	2.28 m	ca. 2.10 m
4′	3.70 m	4.89 dd (5.2, 10.1)	3.80 m	4.89 dd (4.9, 10.3)	1.55 m	ca. 1.50 m	1.60 m	ca. 1.50 m
5′a	3.61 m	3.69 dd (5.8, 9.8)	3.70 m	3.68 dd (5.4, 9.9)	3.61 m	3.54 m	3.59 m	3.55 m
5′b	n/a	3.59 dd (4.6, 9.8)	n/a	3.56 dd (4.9, 9.9)	3.42 m	3.40 m	3.45 m	3.42 m
6′	4.10 m	n/a	4.00 m	n/a	3.61 m	3.95 m	3.59 m	3.93 m
7′	6.05 dd (3.1, 15.5)	5.93 dd (3.3, 15.6)	6.05 dd (3.2, 15.6)	5.92 dd (3.2, 15.5)	6.01 dd (2.6, 15.4)	5.96 dd (3.1, 15.4)	6.00 dd (2.5, 15.5)	5.94 dd (3.1, 15.4)
8′	7.7 dd (11.3, 15.5)	7.54 dd (11.5, 15.6)	7.75 dd (11.4, 15.6)	7.54 dd (11.2, 15.5)	7.60 dd (11.6, 15.4)	7.67 dd (11.5, 15.4)	7.65 dd (11.5, 15.5)	7.68 dd (11.5, 15.4)
9′	6.67 dd (11.3, 11.3)	6.61 dd (11.5, 11.5)	6.67 dd (11.4, 11.4)	6.59 dd (11.2, 11.2)	6.67 dd (11.6, 11.6)	6.65 dd (11.5, 11.5)	6.66 dd (11.5, 11.5)	6.67 dd (11.5, 11.5)
10′	5.78 d (11.3)	5.78 d (11.5)	5.77 d (11.4)	5.79 d (11.2)	5.78 d (11.6)	5.78 d (11.5)	5.79 d (11.5)	5.77 d (11.5)
12′	1.01 d (6.4)	1.06 d (6.9)	1.03 d (6.5)	1.08 d (6.8)	1.11 d (6.9)	1.12 d (6.9)	1.12 d (6.9)	1.09 d (6.9)
13′	3.70 m	5.04 dq (6.4, 6.4)	3.80 m	4.91 dq (3.5, 6.5)	3.61 m	4.89 dq (3.8, 6.5)	3.59 m	5.02 m*
14′	1.20 d (6.0)	1.17 d (6.4)	1.17 d (6.4)	1.20 d (6.5)	1.16 d (6.4)	1.21 d (6.5)	1.19 d (6.0)	1.19 d (6.5)
CH_3COO	—	2.04 s	—	2.06 s		2.06 s		2.04 s
		2.08 s		2.08 s		2.07 s		2.06 s
		0.10 s		2.10 s		2.14 s		2.13 s

*Overlaps with H8 and H2′.

($\Delta\delta \approx 1.2$ ppm) in the *threo* series (C13′*R*), and the C14′ methyl resonance also usually resonates at a higher frequency when C13′ is *R* (see Tables 1 and 2).

When tested in vivo against P388 mouse leukemia,[5] baccharinoids B1-B3 and B7 exhibited the following activities (compound, % T/C, dosage): baccharinoid B1 [**11a**], 175% (0.8 mg/kg); baccharinoid B2 [**12a**], 203% (1.0 mg/kg); baccharinoid B3 [**5a**], 250% (5.0 mg/kg); baccharinoid B7 [**6a**], 196% (0.62 mg/kg).

TABLE 2. ^{13}C-nmr Spectral Data for Baccharinoids B1 [**11a**], B2 [**12a**], B3 [**5a**], and B7 [**6a**]

Position	Compounds			
	B1 [**11a**]	B2 [**12a**]	B3 [**5a**]	B7 [**6a**]
2	79.3	79.3	79.3	79.2
3	34.9	34.9	35.0	35.0
4	74.1	74.2	74.3	74.3
5	49.4	49.4	49.4	49.4
6	45.3	45.3	45.7	45.7
7	30.7	30.8	30.8	30.9
8	68.3	68.3	68.2	68.2
9	143.0	142.9	143.3	143.3
10	120.8	120.9	120.6	120.9
11	67.3	67.3	67.2	67.2
12	65.3	65.3	65.1	65.1
13	47.9	47.9	47.8	47.8
14	7.0	7.0	7.1	7.1
15	64.1	64.1	64.7	64.8
18	18.8	18.8	18.8	18.8
1′	172.7	172.7	172.6	172.5
2′	38.3	38.4	76.9	76.9
3′	32.4	32.6	35.0	34.8
4′	73.1	73.2	31.0	30.8
5′	74.1	74.5	68.3	67.9
6′	85.8	84.6	82.6	83.9
7′	139.8	139.5	138.8	139.1
8′	126.5	126.6	127.0	126.8
9′	144.1	144.2	143.8	143.7
10′	117.6	117.4	117.3	117.6
11′	166.8	166.8	166.8	166.7
12′	15.1	14.9	16.1	16.1
13′	71.1	70.0	69.7	70.7
14′	18.6	17.8	18.1	18.3

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Fisher-Johns hot stage melting point apparatus and are uncorrected. Ir spectra were determined in $CHCl_3$ or CH_2Cl_2 on a Perkin-Elmer Model 183 spectrometer. Uv spectra were determined in MeOH on a Perkin-Elmer Model 552 spectrophotometer. Optical rotations were determined on a Perkin-Elmer 241 automatic polarimeter. Nmr spectra were determined in $CDCl_3$ on an IBM SY-200 MHz spectrometer with TMS as an internal standard; ^{13}C nmr were assigned by using INEPT and by comparison of chemical shift data with those in the literature. Mass spectra were determined on a VG 7070EQ mass spectrometer in the negative ion mode with NH_3 as a reagent gas or in the positive ion mode with CH_4 as the reagent gas.

[5]Antileukemic activity was assayed under the auspices of the National Cancer Institute by the procedures described by Geran *et al.* (8).

Tlc was performed on precoated tlc plates of Si gel 60F-254 (0.2 mm). Visualization was done by viewing the developed plates under short wavelength uv light and by spraying with vanillin/H_2SO_4. The chromatotron (Harrison Research Laboratories) Model 7942 was used for preparative tlc with the plates of 1, 2, or 4 mm thickness prepared according to instruction in the manual using E. Merck Si gel or alumina on regular glass circular discs.

Hplc was done on an Altex Model 302 gradient hplc. An analytical Spherisorb amino (5 μ, 25 cm×4.6 mm) and a semipreparative Spherisorb amino (5 μ, 25 cm×10 mm) columns were used.

METHANOLYSIS OF BACCHARINOIDS.—To a solution of *n*-butyllithium (8 equiv) in dry MeOH at ca. −70° under argon was added the baccharinoid. The resulting solution was stirred at room temperature for ca. 5.5 h, poured onto a small column of Si gel, and washed with 20% MeOH in EtOAc. The filtrate was evaporated, and the residue was subjected to preparative tlc on Si gel (8% MeOH/$CHCl_3$) to give the trihydroxy trichothecene which was identical in mp, $[\alpha]_D$, nmr, and mass spectra with 8β-hydroxyverrucarol [**7**] reported earlier (1).

TLC DATA.—Table 3 gives the tlc data for the baccharinoids B1 [**11a**], B2 [**12a**], B3 [**5a**], and B7 [**6a**].

TABLE 3. Rf Values for Baccharinoids B1, B2, B3, and B7 on Si Gel Tlc

Baccharinoid	Rf Values in Solvents		
	A[a]	B[b]	C[c]
B1 [**11a**]	0.50	0.18	0.51
B2 [**12a**]	0.48	0.19	0.47
B3 [**5a**]	0.52	0.30	0.58
B7 [**6a**]	0.54	0.29	0.55

[a]5% MeOH/CH_2Cl_2.
[b]EtOAc.
[c]40% iPrOH/hexane.

ACETYLATION OF BACCHARINOIDS.—The baccharinoid dissolved in CH_2Cl_2 (usually 5-10 mg in 1 ml of CH_2Cl_2) was mixed with an excess amount of Ac_2O (ca. 5 equiv) and triethylamine (ca. 10 equiv) with a catalytic amount of *N,N*-dimethylaminopyridine (DMAP) added. After 15-20 min, the reaction mixture was concentrated in vacuo, and the product was purified on the chromatotron (1 mm SiO_2 plate; 0-2% MeOH/CH_2Cl_2).

ISOLATION OF BACCHARINOIDS B3 [**5a**] AND B7 [**6a**].—Fraction VIII (3) (162 g) was dissolved in 1 liter CH_2Cl_2, and a 2.5% solution of aqueous NaOH was carefully poured on top of the organic phase. The phases were gently stirred and allowed to separate. The upper aqueous layer was decanted, and the process repeated three times. After separation of the organic phase, the aqueous layer was washed carefully with CH_2Cl_2 twice. The CH_2Cl_2 layers were combined, dried ($MgSO_4$), and concentrated in vacuo to give 60.7 g of material.

The crude sample was divided into two parts. Each one (ca. 30 g) was subjected to filtration chromatography on alumina (140 g, activity III) by using (A) 4 liters of 15% iPrOH in hexane, (B) 1 liter of 100% iPrOH, and (C) 1 liter of 100% MeOH to give three fractions: A (15 g), B (32 g), and C (3 g), respectively. A tlc analysis of fraction A indicated no trichothecenes were present.

The crystallization of fraction B from EtOAc/hexane gave 3.5 g of B7 [**6a**]. Recrystallization from EtOH yielded 2.5 g of pure B7 [**6a**]: mp 229-231°; $[\alpha]^{25}D$+150° (*c* 0.66, CH_2Cl_2); uv (EtOH) λ max (ε) 263 nm (20,100); ir ($CHCl_3$) 3590, 2450, 1720, 1650, 1605 cm^{-1}; ms (ci, CH_4) calcd. for *m/z* $C_{29}H_{40}O_{10}$+H 549.2704, found 549.2681.

A portion (100 mg) of the mother liquor of fraction B was subjected to preparative tlc (2mm SiO_2 plate; two developments with 10% MeOH/$CHCl_3$). Extraction of the bands with 20% MeOH/CH_2Cl_2 gave the fractions C (60 mg) and D (40 mg) which contained baccharinoids B7 [**6a**] and B3 [**5a**], respectively. Recrystallization of fraction D from Me_2CO/hexane gave pure baccharinoid B3 [**5a**]: mp 172-180°; $[\alpha]^{25}D$+164° (*c* 0.58, CH_2Cl_2; uv (EtOH) λ max (ε) 263 nm (20,200); ir ($CHCl_3$) 3590, 3450, 1715, 1640, 1600 cm^{-1}; ms (ci, CH_4) calcd. for *m/z* $C_{29}H_{40}O_{10}$+H 549.2704, found 549.2687.

PREPARATION OF **10** FROM B7 [**6a**].—A solution of baccharinoid B7 [**6a**], (120.0 mg, 0.22 mmol) in absolute EtOH (40 ml) was hydrogenated at atmospheric pressure using 10% palladium on charcoal

(17.6 mg) as catalyst. The reaction was followed by hydrogen uptake and by the disappearance of uv absorption. When two equivalents of hydrogen were taken up (10-15 min), the catalyst was removed by filtration through Celite, and the solvent was evaporated. The resulting colorless solid, tetrahydro B7 [**9b**], was dried and used without further purification: ms (ci, CH_4) calcd. for *m/z* $C_{29}H_{44}O_{10}$+H 553.3012, found 553.3018.

To vacuum-dried tetrahydro-B7 [**9b**] (107.0 mg, 0.193 mmol), prepared in the above manner, in 7 ml of dry CH_2Cl_2 was added anhydrous NaOAc (11.5 mg, 0.140 mmol) and pyridinium chlorochromate (135 mg, 0.626 mmol). The mixture was stirred for 4 h. The reaction mixture was filtered, and the solids were washed with 2 ml of CH_2Cl_2. The combined filtrates were extracted with 10 ml of H_2O, and the solvent was evaporated in vacuo. Preparative tlc on Si gel, developed with 6% MeOH in $CHCl_3$, gave a colorless glass (70 mg, 66%). Crystallization from Me_2CO/Et_2O afforded **10**: mp 152-154°; uv (EtOH) λ max (ε) 226 nm (8300); ir ($CHCl_3$) 3530, 1730, 1685 cm^{-1}; ms (ci, CH_4) calcd. for *m/z* $C_{29}H_{40}O_{10}$+H 549.2700, found 549.2703.

PREPARATION OF **10** FROM B3 [**5a**].—By the same procedure as that described for the hydrogenation of B7 [**6a**], B3 [**5a**] afforded tetrahydro B3 [**9a**]: ms (ci, CH_4) calcd. for *m/z* $C_{29}H_{44}O_{10}$+H 553.3012, found 553.3027.

By the same procedure as that described for **9b**, tetrahydro B3 [**9a**] (105 mg, 0.189 mmol) gave **10** (65 mg, 63%), identical to that obtained from **9b** in mp, ir, uv, nmr, and ms.

ISOLATION OF BACCHARINOIDS B1 [**11a**] AND B2 [**12a**].—A portion (200 mg) of fraction IX-D4 (3) was injected on a semiprep amino hplc column with 1.5% MeOH/CH_2Cl_2. A total of 15 injections were made in amounts ranging from 12 to 18 mg each to give two fractions, a and b, containing baccharinoids B1 [**11a**] and B2 [**12a**], respectively. Fraction a was crystallized from CH_2Cl_2/hexane. Recrystallization from EtOAc/hexane gave 60 mg of pure baccharinoid B1 [**11a**]: mp 162-164°; $[\alpha]^{24}D$+100 (*c* 1.4, CH_2Cl_2); uv (EtOH) λ max (ε) 263 nm (20,100); ms (ci, CH_4) calcd. for *m/z* $C_{29}H_{40}O_{10}$+H 549.2704, found 549.2681.

Crystallization and recrystallization of fraction b from CH_2Cl_2/Et_2O gave 100 mg of pure baccharinoid B2 [**12a**]: mp 177-180°; $[\alpha]^{24}D$+116° (*c* 1.0, CH_2Cl_2); uv (EtOH) λ max (ε) 263 nm (20,100); ms (ci, CH_4) calcd. for *m/z* $C_{29}H_{40}O_{10}$+H 549.2704, found 549.2681.

HYDROGENATION OF BACCHARINOID B1 [**11a**].—Baccharinoid B1 [**11a**] (25 mg) in 25 ml of absolute EtOH was hydrogenated by a procedure similar to that described for **9b**, to give tetrahydro B1 [**13a**] (22.5 mg, 87%) as a colorless solid: ir (CH_2Cl_2) 3460, 2890, 1735, 1725 cm^{-1}; ^1H nmr ($CDCl_3$) δ 0.79 (3H, s, 14-H), 1.00 (3H, d, *J*=6.2 Hz, 12′-H), 1.09 (3H, d, *J*=6.6 Hz, 14′-H), 1.79 (3H, s, 16-H), 1.98 (1H, ddd, *J*=4.4, 5.0, and 15.4 Hz, 3β-H), 2.44 (1H, dd, *J*=8.1 and 15.4 Hz, 3α-H), 2.83, 3.12 (1H each, AB pattern, *J*=3.9 Hz, 13-H), 3.66 (1H, d, *J*=5.1 Hz, 11-H), 3.81 (1H, d, *J*=5.0 Hz, 2-H), 3.90, 4.25 (1H each, AB pattern, *J*=12.3 Hz, 15-H), 5.45 (1H, d, *J*=5.1 Hz, 10-H), 5.65 (1H, dd, *J*=4.4 and 8.1 Hz, 4-H); ms (negative ion ci, NH_3) calcd. for *m/z* $C_{29}H_{44}O_{10}$ 552.2934, found 552.2887.

HYDROGENATION OF BACCHARINOID B2 [**12a**].—Hydrogenation of baccharinoid B2 [**12a**] by a similar procedure as described above, gave 91% of tetrahydro B2 [**13b**]; ir (CH_2Cl_2) 3480, 2830, 1735, 1725 cm^{-1}; ^1H nmr ($CDCl_3$) δ 0.81 (3H, s, 14-H), 0.96 (3H, d, *J*=6.0 Hz, 12′-H), 1.09 (3H, d, *J*=6.0 Hz, 14′-H), 1.79 (3H, s, 16-H), 2.83, 3.12 (1H each, AB pattern, *J*=3.9 Hz, 13-H), 3.65 (1H, d, *J*=4.8 Hz, 11-H), 3.82 (1H, d, *J*=5.0 Hz, 2-H), 3.92, 4.25 (1H each, AB pattern, *J*=12.2 Hz, 15-H), 5.45 (1H, d, *J*=4.8 Hz, 10-H), 5.65 (1H, dd, *J*=4.0, 7.8 Hz, 4-H); ms (negative ion ci, NH_3) calcd. for *m/z* $C_{29}H_{44}O_{10}$ 552.2934, found 552.2906.

OXIDATION OF TETRAHYDRO B1 [**13a**].—Tetrahydro B1 [**13a**] (14.0 mg) was oxidized with PCC by a similar procedure as described for **9b** to yield the 8,13′-diketo derivative of baccharinoid B1 [**14**] (7.0 mg, 50%): ir (CH_2Cl_2) 3600, 2880, 1760, 1725, 1630, 1170 cm^{-1}; ^1H nmr ($CDCl_3$) δ 0.79 (3H, s, 14-H), 0.96 (3H, d, *J*=6.5 Hz, 12′-H), 1.81 (3H, s, 16-H), 2.14 (3H, s, 14′-H), 2.20 (2H, m, 3β-H and 3′-H), 2.53 (1H, dd, *J*=8.1 and 15.8 Hz, 3α-H), 2.57, 2.85 (1H each, AB pattern, *J*=15.3 Hz, 7-H), 2.84, 3.13 (1H each, AB pattern, *J*=3.9 Hz, 13-H), 3.36 (1H, dd, *J*=6.4 and 10.1 Hz, 5′B-H), 3.54 (1H, dd, *J*=3.2 and 10.1 Hz, 5′A-H), 3.76 (2H, m, 4′-H and 6′-H); 3.90 (1H, d, *J*=5.0 Hz, 2-H), 3.97 (1H, d, *J*=5.7 Hz, 11-H), 4.03, 4.27 (1H each, AB pattern, *J*=12.4 Hz, 15-H), 5.75 (1H, dd, *J*=4.0 and 8.1 Hz, 4-H), 6.49 (1H, d, *J*=5.7 Hz, 10-H); ms (negative ion ci, NH_3) calcd. for *m/z* $C_{29}H_{40}O_{10}$ 548.2621, found 548.2602.

OXIDATION OF TETRAHYDRO B2 [**13b**].—PCC oxidation of tetrahydro B2 [**13b**] by the same procedure as that described for **13a** gave its 8,13′-diketo derivative which was identical to that obtained from tetrahydro B1 [**13a**] as shown by ^1H nmr and ms.

SINGLE CRYSTAL X-RAY DIFFRACTION ANALYSIS OF BACCHARINOID B2 TRIACETATE [**12b**].—Distorted cube-shaped crystals of **12b** were obtained by slow evaporation of a CH_2Cl_2/hexane solution. A 0.3×0.36×0.46 mm crystal was placed on an Enraf-Nonius CAD-4 diffractometer equipped with an incident beam graphite monochromator and a Mo X-ray source (MoKα, λ=0.71069 Å). The unit cell parameters were obtained from 25 reflections in the θ range of 12.7-18.2°: triclinic space group, P1, a=8.383 (1), b=10.533 (2), c=11.141 (3) Å, α=68.90 (2), β=80.07 (1), γ=72.09 (1)°, Z=1, P X-

TABLE 4. Fractional Coordinates and Equivalent Isotropic Temperature Parameters (Å^2) for Baccharinoid B2 Triacetate [**12b**]

Atom	*x*	*y*	*z*	B(eq)
O1	−0.4339	0.2187	0.8736	3.8(2)
O2	−0.3472(4)	0.4909(3)	0.9654(3)	4.5(2)
O3	−0.4389(4)	0.6659(3)	0.6554(3)	3.9(2)
O4	−0.3026(4)	0.6774(3)	0.4600(3)	4.6(2)
O5	0.4487(4)	0.9365(3)	0.2068(3)	4.6(2)
O6	0.5295(4)	1.1191(3)	0.2077(3)	6.0(3)
O7	0.2195(4)	0.7754(3)	0.2888(3)	3.7(2)
O8	0.1104(4)	0.5531(3)	0.2751(3)	3.4(2)
O9	0.1677(4)	0.5379(4)	0.0758(3)	6.9(3)
O10	0.0432(3)	0.3285(3)	0.5689(3)	3.1(2)
O11	0.1061(4)	0.2364(3)	0.4093(3)	5.2(2)
O12	0.1319(4)	−0.0612(3)	0.8746(3)	3.6(2)
O13	0.3441(4)	0.0077(3)	0.7441(3)	6.2(2)
C2	−0.4833(5)	0.3535(4)	0.8943(4)	4.0(3)
C3	−0.5643(5)	0.4753(5)	0.7801(4)	4.1(3)
C4	−0.4162(5)	0.5164(4)	0.6872(4)	3.5(3)
C5	−0.2528(4)	0.4270(3)	0.7603(3)	2.8(2)
C6	−0.1876(4)	0.2847(4)	0.7298(3)	2.7(2)
C7	−0.0489(5)	0.1769(4)	0.8160(3)	2.9(2)
C8	0.0153(5)	0.0417(4)	0.7817(4)	3.1(2)
C9	−0.1242(5)	−0.0192(4)	0.7823(4)	3.5(3)
C10	−0.2798(5)	0.0635(4)	0.7647(4)	3.5(3)
C11	−0.3352(5)	0.2159(4)	0.7550(3)	3.2(3)
C12	−0.3271(4)	0.3919(4)	0.8986(4)	3.2(3)
C13	−0.2390(6)	0.3483(4)	1.0141(4)	4.3(3)
C14	−0.1196(5)	0.5067(4)	0.7342(4)	3.5(3)
C15	−0.1253(5)	0.3094(4)	0.5872(3)	3.0(3)
C16	−0.0782(7)	−0.1725(5)	0.7954(5)	5.1(4)
C17	0.5620(5)	1.0091(4)	0.1880(4)	4.1(3)
C18	0.7276(6)	0.9347(6)	0.1409(5)	5.5(4)
C19	0.0709(5)	0.5449(4)	0.1674(4)	4.3(3)
C20	−0.1080(7)	0.5410(7)	0.1795(6)	6.2(5)
C21	0.2946(5)	−0.0694(4)	0.8414(4)	4.0(3)
C22	0.4034(7)	−0.1813(5)	0.9439(6)	5.8(4)
C1′	0.1457(5)	0.2875(4)	0.4763(4)	3.3(3)
C2′	0.3159(5)	0.3089(4)	0.4704(4)	3.5(3)
C3′	0.3807(4)	0.3873(4)	0.3353(3)	3.0(2)
C4′	0.2852(5)	0.5428(4)	0.2833(4)	3.1(3)
C5′	0.2833(6)	0.6324(4)	0.3628(4)	3.7(3)
C6′	0.2181(5)	0.8737(4)	0.3504(4)	3.8(3)
C7′	0.0483(5)	0.9269(4)	0.4095(4)	4.2(3)
C8′	−0.0806(5)	0.8735(4)	0.4280(4)	3.8(3)
C9′	−0.2420(5)	0.9311(4)	0.4885(4)	4.4(3)
C10′	−0.3682(6)	0.8735(4)	0.5381(4)	4.1(3)
C11′	−0.3640(5)	0.7315(4)	0.5417(4)	3.7(3)
C12′	0.5681(5)	0.3719(5)	0.3316(5)	4.4(3)
C13′	0.2779(5)	0.9961(4)	0.2509(4)	4.2(3)
C14′	0.1721(7)	1.0761(5)	0.1378(5)	5.9(4)

ray=1.286 g cm^{-3}. X-ray intensity data were measured over the range θ=2-25°, with a θ speed of 0.91-8.24° min^{-1} and scan range of 1.0+0.35 tan θ. The scans were recorded as 96 step profiles and was subsequently processed with a modified Lehmann-Larson procedure (9,10). The crystal was automatically recentered at 500 reflection intervals, and 7 standards were measured every 2 h; 3395 total data measured, 3056 unique data, 2670 data $\geq 3\sigma$ above background. The structure was solved with some difficulty by the direct methods program MITHRIL (11) [part of the TEXSAN crystallographic system (12)]. Structure refinement was by full-matrix least-squares with individual anisotropic temperature factors for carbon and oxygen and isotropic terms for hydrogens and included a connection for isotropic secondary extinction. Most of the hydrogen atoms were intially positioned from the geometry of the C-O framework; the methyl hydrogens were located from a difference map. The final R and weighted R factors are 0.031 and 0.019. All calculations were performed on a MicroVax II computer with the TEXSAN system (12). Atomic coordinates from the non-hydrogen atoms are given with their standard deviations in Table 4.[6]

TABLE 5. Fractional Coordinates and Equivalent Isotropic Temperature ($Å^2$) for Baccharinoid B7 [**6a**]

Atom	*x*	*y*	*z*	B(eq)
O1	0.4685(2)	0.7372(4)	0.2788(1)	2.8
O2	0.3702(2)	0.8409(5)	0.4713(1)	3.6
O3	0.2370(2)	0.4621(4)	0.4025(2)	3.4
O4	0.0924(2)	0.3562(6)	0.3002(2)	5.1
O5	−0.4399(2)	0.3295(5)	0.1980(2)	4.8
O6	−0.0709(2)	1.0860(4)	0.1133(2)	4.6
O7	−0.2530(2)	0.5466(4)	0.2427(2)	3.3
O10	0.0829(2)	0.8292(4)	0.1414(1)	3.0
O11	−0.3814(3)	0.1422(7)	0.3263(3)	4.2
O12	0.3702(2)	1.2458(4)	0.1168(2)	3.7
C2	0.4541(2)	0.6948(6)	0.3631(2)	3.1
C3	0.4064(3)	0.4987(6)	0.3670(2)	3.3
C4	0.2832(3)	0.5257(6)	0.3328(2)	2.9
C5	0.2642(2)	0.7440(6)	0.3171(2)	2.5
C6	0.2715(2)	0.7902(5)	0.2211(2)	2.4
C7	0.2827(3)	1.0030(5)	0.2068(2)	2.6
C8	0.2943(3)	1.0539(6)	0.1160(2)	2.8
C9	0.3682(3)	0.9213(6)	0.0845(2)	3.0
C10	0.4030(3)	0.7617(6)	0.1262(2)	2.9
C11	0.3747(3)	0.6956(5)	0.2071(2)	2.6
C12	0.3681(3)	0.8200(6)	0.3794(2)	2.8
C13	0.3842(3)	1.0067(6)	0.4203(2)	3.7
C14	0.1625(3)	0.8162(6)	0.3363(2)	3.2
C15	0.1753(3)	0.7050(6)	0.1514(2)	2.8
C16	0.3989(3)	0.9769(8)	0.0031(3)	4.5
C1′	−0.0085(3)	0.7696(7)	0.0865(2)	3.2
C2′	−0.1021(3)	0.9058(6)	0.0772(2)	3.5
C3′	−0.1881(3)	0.8259(6)	0.1181(2)	3.1
C4′	−0.1460(3)	0.8111(7)	0.2171(2)	3.7
C5′	−0.2239(3)	0.7380(7)	0.2662(3)	3.9
C6′	−0.2866(3)	0.4457(6)	0.3093(2)	3.1
C7′	−0.1935(3)	0.3924(7)	0.3848(2)	3.7
C8′	−0.0927(3)	0.3760(7)	0.3786(3)	4.0
C9′	−0.0032(3)	0.3292(8)	0.4517(3)	4.5
C10′	0.1012(3)	0.3308(8)	0.4534(3)	4.5
C11′	0.1393(3)	0.3789(7)	0.3764(3)	3.7
C12′	−0.2891(3)	0.9459(7)	0.0889(3)	4.1
C13′	−0.3461(3)	0.2711(7)	0.2646(2)	3.6

[6]Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, UK.

SINGLE CRYSTAL X-RAY DIFFRACTION ANALYSIS OF BACCHARIN B7 [**6a**].—Crystals suitable for X-ray study were grown by slow evaporation of a solution of the compound in $CHCl_3$. $C_{29}H_{40}O_{10}$, M=548.6, monoclinic, space group $P2_1$, a=12.888 (2), b=7.083 (3), c=15.840 (2) Å, β=105.57 (3)°, U=1393 Å^3, D_{obs}=1.30 (1) g/cm^3, D_x=1.308 g/cm^3, Z=2, F(000)=588. The crystal system and space group were determined from 25° precession photographs taken with MoKα radiation. Unit cell dimensions were obtained by a least-squares fit to the observed values of ±2θ for 20 strong general reflections measured with CuKα radiation (λ=1.5418 Å) on a Picker four-circle diffractometer operated under the control of an XDS Sigma 2 computer. Intensity data were measured from a single-crystal plate 0.4×0.7×0.06 mm, mounted with the b* axis parallel to the φ axis of the diffractometer. A single quadrant of reciprocal space was surveyed with graphite-monochromatized CuKα radiation to 2θ=120°. The θ/2θ scan method was used with fixed 2° scan widths and a scan speed of 2°/min. Background intensity was measured at either end of the scan ranges for 15 s with both crystal and counter stationary. Scintillation counting was used with pulse-height analysis. Scattered intensity significantly above background [I>3σ(I)] was found at 2126 of the 2262 independent locations surveyed. Stability of the experimental conditions was monitored by measurement of the intensities of two reference reflections after every 50 measurement cycles. The r-m-s deviation from the mean intensity was <1% in each case. No absorption correction was applied. The phase problem was solved by routine application of the program MULTAN (13) using the 300 E(hkl)>1.40. Refinement was by the block-diagonal least-squares method with anisotropic thermal parameters adopted for the non-hydrogen atoms. All hydrogen stoms with the exception of that attached to O(2′) were located from three-dimensional difference electron-density maps, and their positions were optimized by the assumption of standard geometries (C-H 1.08 Å, H-C-H 109.5°, etc.). These atoms were included in the least-squares calculations with fixed positions and with fixed isotropic B values, of 4.0 Å^2 when attached to C and 5.0 Å^2 when attached to O. The function minimized was $\Sigma\omega(|F_o|-|F_c|)^2$ with weights assigned in a standard manner (14). Convergence was assumed with the largest shift-to-error ratio being 0.19 and the average 0.08. The conventional unweighted and weighted residuals were 0.040 and 0.053, respectively. A final difference electron-density map contained no structurally significant information and had no density >0.3e/Å^3. The scattering functions used were taken from Cromer and Waber (15) and Stewart, Davidson, and Simpson (16). All computations with the exception of MULTAN and ORTEP (17) were carried out using programs written in this laboratory for the XDS Sigma 2 computer. Atomic coordinates for the non-hydrogen atoms are given with their standard deviations in Table 5. A view of the molecule in the conformation found in the crystal is shown as Figure 1. Although the absolute configuration of the molecule has not been established by the X-ray analysis, the Figure is drawn to conform to that found by X-ray analysis of the *p*-iodobenzenesulfonate of verrucarin A (18). Listing of observed and calculated structure amplitudes are available upon request from R.F. Bryan.[6]

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