(6) T. D. Turner and S. L. Wightman, *J. Chromatogr.*, **32**, 315 (1968), and references cited therein.
(7) R. Hakanson and A. L. Ronnberg, *Anal. Biochem.*, **54**, 353 (1973); *Chem. Abstr.*, **79**, 63336r (1973).
(8) L. Seekles, *Recl. Trav. Chim. Pays-Bas*, **42**, 706 (1923).
(9) W. Treibs, H. M. Barchet, G. Bach, and W. Kirchhof, *Justus Leibigs Ann. Chem.*, **574**, 54 (1951).
(10) Catalin Covalent Models obtained from Catalin, Ltd., Waltham Abbey, Essex, England.
(11) After this paper was submitted for publication an isoindole intermediate was also suggested to account for the products from phthalaldehyde, *n*-propylamine, and 2-mercaptoethanol: S. Simons, Jr., and D. F. Johnson, *J. Am. Chem. Soc.*, **98**, 7098 (1976).
(12) R. Bonnett and R. F. C. Brown, *J. Chem. Soc., Chem. Commun.*, 393 (1972).
(13) G. M. Priestly and R. N. Warrener, *Tetrahedron Lett.*, 4295 (1972).
(14) R. Kreher and J. Seubet, *Z. Naturforsch. B*, **20**, 75 (1965).
(15) J. Bornstein, D. E. Remy, and J. E. Shields, *J. Chem. Soc., Chem. Commun.*, 1149 (1972).
(16) J. Bornstein, D. E. Remy, and J. E. Shields, *Tetrahedron Lett.*, 4247 (1974).
(17) D. F. Veber and W. Lwowski, *J. Am. Chem. Soc.*, **86**, 4152 (1964).
(18) R. I. Fryer, B. Brust, J. V. Early, and L. H. Sternbach, *J. Chem. Soc. C*, 366 (1967).
(19) A precedent for this reaction is found in the preparation of benzamidine hydrochloride by the reaction of alcoholic ammonia on the imino ester hydrochloride obtained from the reaction of benzonitrile, hydrogen chloride, and ethanol: see A. W. Dox in "Organic Syntheses", Collect. Vol. I, 2nd ed, Wiley, New York, N.Y., 1958, p 6; see also A. Pinner, *Ber.*, **16**, 1643 (1883); **17**, 178 (1884).
(20) C. Aso and S. Tagami, *Am. Chem. Soc., Div. Polym. Chem., Prepr.*, **8**, 906 (1967); *Macromolecules*, **2**, 414 (1969).
(21) C. Graebe and P. Pictet, *Justus Liebigs Ann. Chem.*, **247**, 303 (1888).
(22) R. E. Ludt and C. R. Hauser, *J. Org. Chem.*, **36**, 1607 (1971); A. Rosenthal and M. R. S. Weir, *J. Org. Chem.*, **28**, 3025 (1963).
(23) J. Thiele and J. Schneider, *Justus Liebigs Ann. Chem.*, **369**, 287 (1909).

Isolation of Potent New Antileukemic Trichothecenes from *Baccharis megapotamica*[1,2]

S. Morris Kupchan,[16] David R. Streelman, Bruce B. Jarvis,[3] Richard G. Dailey, Jr., and Albert T. Sneden*

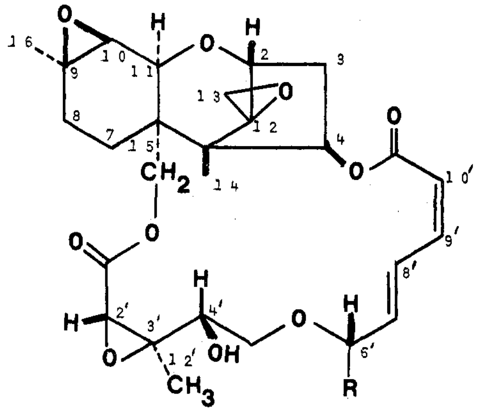
Department of Chemistry, University of Virginia, Charlottesville, Virginia 22901

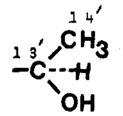
Received June 14, 1977.

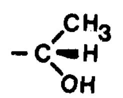
The isolation and structural elucidation of the new potent antileukemic trichothecenes baccharin (1), baccharinol (2), isobaccharinol (3), and isobaccharin (4) are reported. Baccharinol (2) and isobaccharinol (3) were shown to be esters of 8β-hydroxyverrucarol (9) by hydrolysis to **9** and the dimethyl esters **5** and **11**. Hydrolysis of baccharin (1) and isobaccharin (4) gave **6** and esters **5** and **11**. Conversion of **2** and **3** to the common intermediate **13** demonstrated that **4** and **3** were the C-13′ epimers of **1** and **2**, respectively.

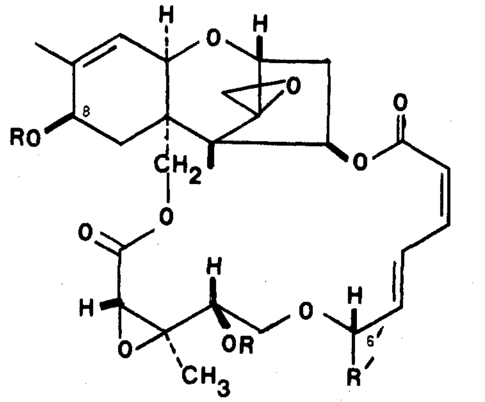
In the course of a continuing search for tumor inhibitors of plant origin, an alcoholic extract of *Baccharis megapotamica* Spreng (Asteraceae)[4] was found to show significant activity in vivo against P-388 leukemia in mice (PS)[5] and in vitro against cells derived from human carcinoma of the nasopharynx (KB). A preliminary communication[6] described the structural elucidation of the potent antileukemic trichothecene triepoxide baccharin (1). It is the purpose of this paper to present in detail the isolation and structural elucidation of baccharin (1), as well as the new potent antileukemic principles baccharinol (2), isobaccharinol (3), and isobaccharin (4).[7]

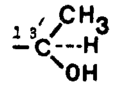
Fractionation of the alcohol extract, guided by a combination of P-388 in vivo assay in mice and KB testing in vitro, revealed that the inhibitory activity was concentrated, suc-

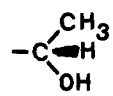


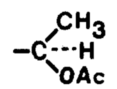
1, R = 

4, R = 



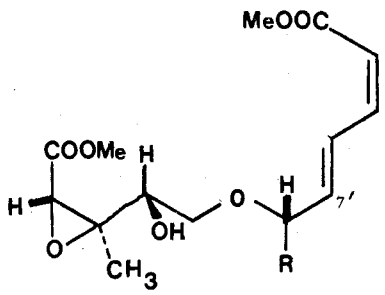
2, R = H ; R′ = 

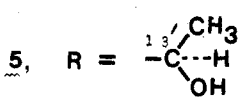
3, R = H ; R′ = 

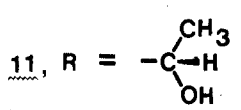
7, R = Ac ; R′ = 

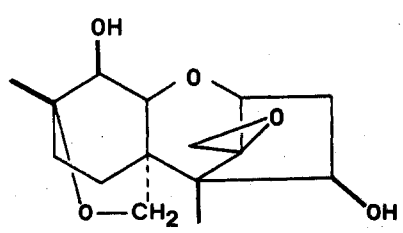
cessively, in the ethyl acetate layer of an ethyl acetate–water partition and in the aqueous methanol layer of a 10% aqueous methanol–petroleum ether partition. The aqueous methanol-soluble material, following filtration through alumina, was submitted to column chromatography on alumina. Elution with 10% methanol in ether gave a fraction containing baccharin (1) and isobaccharin (4), as well as other related trichothecene epoxides. Further column chromatography on silica gel, with methanol in chloroform as eluent, yielded pure baccharin (1), followed by a fraction which, after purification by preparative TLC, gave isobaccharin (4). Elution of the alumina column with methanol gave a residue containing baccharinol (2) and isobaccharinol (3). Rechromatography on silica gel with methanol–ether as eluent, followed by preparative TLC on silica gel, afforded the closely related compounds baccharinol (2) and isobaccharinol (3).

Elemental analysis and high-resolution mass spectrometry established that all four compounds were isomeric, with molecular formula of $C_{29}H_{38}O_{11}$. The ^1H NMR spectrum of each compound contained a pair of doublets (J = 4 Hz) centered at ca. 3.0 ppm corresponding to an exocyclic epoxide, as well as signals characteristic of a dienoate ester. Methanolysis of 1 gave a dimethyl ester, later identified as **5**, and a 15-carbon fragment which was later shown to be **6**, a known compound[8] resulting from intramolecular opening of the 9,10-epoxide by the C-15 hydroxyl.





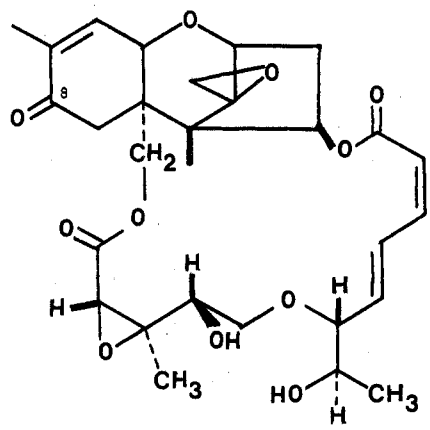




6

Consideration of the above data suggested that these compounds were structurally related to the roridins, a class of macrocyclic diesters of the 12,13-epoxytrichothecene, verrucarol. Confirmation was obtained by the determination of the structure and stereochemistry of baccharin (1) via a direct single-crystal x-ray analysis, the results of which were described in our preliminary communication.[6]

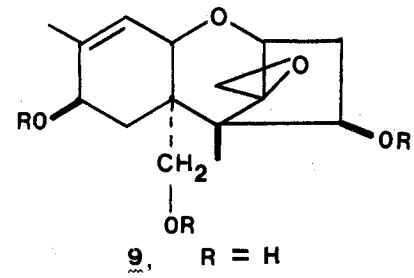
The ^1H NMR spectrum of baccharinol (2) was similar to that of baccharin (1) except that the C-16 methyl at δ 1.37 in 1 was shifted to δ 1.83 in 2 and the C-10 H at δ 3.11 had shifted to δ 5.46, changes which indicated that the 9,10-epoxide in 1



8

was replaced with a carbon–carbon double bond. The last oxygen atom was shown to be present as a hydroxyl group in baccharinol (2), since acetylation of 2 in pyridine–acetic anhydride gave triacetate **7**. The allylic nature of the hydroxyl was demonstrated by oxidation of 2 to give the unsaturated ketone **8**. The shift of the C-10 hydrogen resonance from δ 5.46 in 2 to δ 6.63 in the ^1H NMR spectrum of 8 confirmed the α,β-unsaturated ketone functionality of 8 and therefore showed that the hydroxyl was allylic to the C-9,10 double bond.

Methanolysis of 2 yielded dimethyl ester **5**, identical to that obtained from baccharin (1), and the trihydroxytrichothecene **9**. Acetylation of 9 produced triacetate **10**, the ^1H NMR



9, R = H

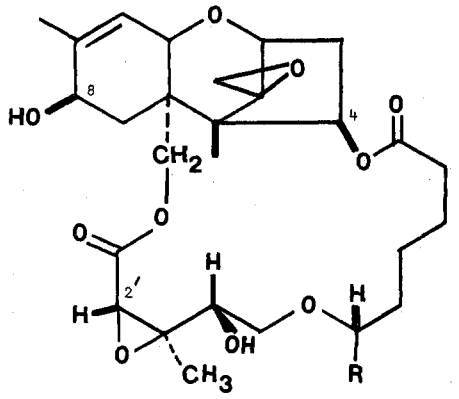
10, R = Ac

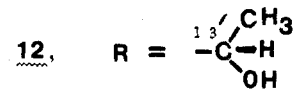
spectrum of which contained a doublet of doublets (J = 8 and 4 Hz) at δ 5.24 assigned to the C-8 hydrogen. Examination of molecular models showed that the observed coupling was more readily accommodated by the postulated stereochemistry with the C-8 oxygen function β. Furthermore, trichothecenes with an α C-8 ester function are known,[9] and the proton in question shows coupling constants of 5.5 and 0.2 Hz in these compounds.[10]

The spectral data of isobaccharinol (3) was nearly identical to that of baccharinol (2), suggesting the possibility that the two differed only in stereochemistry. Methanolysis of 3 gave 8β-hydroxyverrucarol (9), identical to that obtained from 2, and the dimethyl ester **11**. The ^1H NMR spectrum of 11 differed substantially from that of 5 only in the chemical shift of the C-7 hydrogen, which was shifted from δ 5.83 in 5 to δ 5.96 in 11, indicating the site of the postulated stereochemical change was probably at C-6′ or C-13′. Further evidence for a stereochemical change in the vicinity of C-6′ or C-13′ was obtained by a comparison of the ^{13}C NMR spectra of baccharinol (2) and isobaccharinol (3) (Table I). Careful assignment of the carbon spectra, utilizing broad-band and off-resonance decoupling techniques, and the relative wealth of published ^{13}C NMR spectra of related compounds,[11] revealed that all of the corresponding carbon resonances of the two

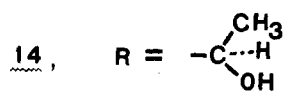
compounds had chemical shifts within a few tenths of 1 ppm of each other with only three exceptions. The exceptions were carbons 6′, 13′, and 14′ which appeared at 86.7, 71.0, and 17.8 ppm, respectively, in **2**, and at 85.2, 69.0, and 15.8 ppm in **3**.

The structure of isobaccharinol (**3**) was finally determined by chemical transformations. Catalytic hydrogenation of **3** gave the tetrahydro derivative **12**. Oxidation of **12** with pyri-



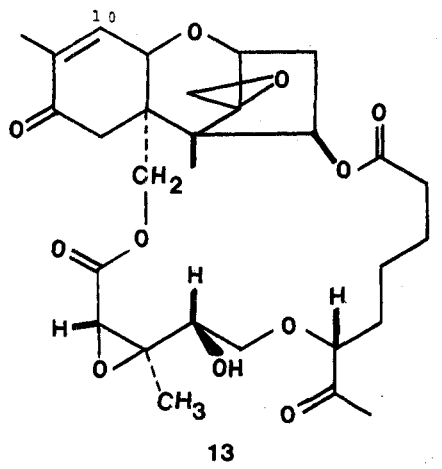


12, R =



14, R =

dinium chlorochromate in dichloromethane yielded the diketo compound **13**. The ^1H NMR spectrum of **13** showed resonances for a vinyl methyl (δ 1.85, br s) and a vinyl proton (δ 6.52, dq, J = 4 and 1.5 Hz) as in **8**, and, in addition, contained a low-field methyl signal at δ 2.17, characteristic of methyl ketones. The infrared spectrum of **13** contained new carbonyl



13

absorptions at 1720 and 1690 cm^{-1}. Hydrogenation of baccharinol (**2**) gave **14** which was oxidized to **13**, identical in all respects to that derived from **3**. The structure of isobaccharinol (**3**), then, was established to be the C-13′ epimer of baccharinol (**2**).

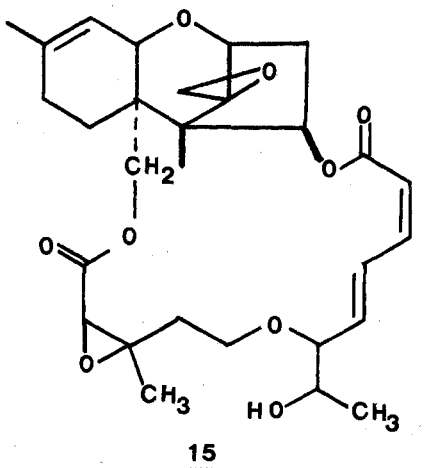
The spectral data of isobaccharin (**4**) suggested that it had the same relationship to baccharin (**1**) as isobaccharinol (**3**) to baccharinol (**2**). Particularly enlightening was the ^{13}C NMR spectra of **4** (Table I), in which the chemical shifts of C-6′, C-13′, and C-14′ were nearly identical to those of **3**, but differed from those of **1** and **2**. Methanolysis of **4** gave **6** and dimethyl ester **11**, the same ester that was obtained from **3**, confirming the structure of isobaccharin (**4**) as the C-13′ epimer of baccharin (**1**).

Table I. ^{13}C NMR Spectra (δ in ppm from Me_4Si)[a]

	Baccharinol (2)	Isobaccharinol (3)	Baccharin (1)	Isobaccharin (4)
C-2	78.6	78.8	78.1	78.2
C-3	34.5	34.5	34.0	34.0
C-4	73.8	73.9	73.8	73.9
C-5	48.9	49.1	48.5	48.5
C-6	44.7	44.8	42.3	42.4
C-7	29.6	29.7	16.7	16.7
C-8	66.7	67.0	25.7	25.8
C-9	143.2	143.3	57.7	57.7
C-10	119.4	119.6	56.9	57.0
C-11	66.4	66.7	66.6	66.7
C-12	65.0	65.4	65.2	65.4
C-13	47.2	47.5	47.1	47.2
C-14	6.5	6.7	6.5	6.6
C-15	64.4	64.6	63.1	63.1
C-16	18.3	18.5	21.6	21.6
C-1′	167.3	167.4	167.4	167.4
C-2′	55.9	56.3	56.0	56.1
C-3′	64.8	65.0	64.4	64.4
C-4′	74.9	75.5	75.3	75.6
C-5′	72.0	72.3	72.1	72.1
C-6′	86.7	85.2	86.8	85.3
C-7′	138.1	138.4	138.2	138.7
C-8′	125.3	125.3	125.1	125.0
C-9′	142.2	142.5	142.6	142.7
C-10′	117.7	117.5	117.4	117.1
C-11′	166.2	166.4	166.3	166.3
C-12′	11.8	11.9	11.6	11.6
C-13′	71.0	69.0	71.0	68.8
C-14′	17.8	15.8	17.7	15.6

[a] Spectra measured in $CDCl_3$ solution containing from 5 to 30% CD_3OD.

Trichothecenes, prior to now, have been observed only as secondary metabolites of imperfect fungi,[12] and have never been found in higher plants. It is noteworthy, then, that we found trichothecenes in large amounts (ca. 0.02% w/w of dried plant material) in two separate collections of *B. megapotamica*, especially in view of their high cyto- and phytotoxicity.[12-14] An examination of the dried plant material revealed no obvious fungal contamination; however, it is possible that the compounds we isolated represent plant-altered fungal products. It also should be noted that, while baccharin (**1**), baccharinol (**2**), isobaccharinol (**3**), and isobaccharin (**4**) all show potent in vivo antileukemic activity against P-388 leukemia in mice,[7] very similar compounds, e.g., roridin D (**15**),[15]



15

show no in vivo activity. The key difference seems to be the presence of an oxygen substitutent in the A ring of the trichothecene nucleus in the baccharinoids. Work is presently in progress in these laboratories to determine more fully the

structural features which are necessary for high in vivo activity.

Experimental Section

General. Melting points were determined on a Mettler Model FP2 hot stage and are uncorrected. Ultraviolet absorption spectra were determined on a Beckman Model DK-2A recording spectrophotometer. Infrared spectra were determined on Perkin-Elmer Model 257 and Model 337 recording spectrophotometers. Nuclear magnetic resonance spectra were determined on a Varian HA-100 spectrometer or a JEOL PS-100 p FT NMR spectrometer interfaced to a Texas Instrument JEOL 980A computer, with tetramethylsilane as an internal standard. Mass spectra were determined on Hitachi Perkin-Elmer Model RMU-6E and AEI Model MS-902 spectrometers. Values of $[\alpha]_D$ were determined on a Perkin-Elmer Model 141 automatic polarimeter. Microanalyses were carried out by Spang Microanalytical Laboratory, Ann Arbor, Mich., and Atlantic Microlab, Inc., Atlanta, Georgia. Petroleum ether refers to the fraction of bp 60–68 °C. All thin-layer chromatography was carried out on prepared plates (E. Merck). Visualization of TLC was effected with short-wavelength UV and concentrated sulfuric acid–vanillin–ethanol (20:1:3) spray.

Extraction and Preliminary Fractionation of *Baccharis megapotamica*. The dried ground twigs and leaves (54 kg) were extracted in 18-kg batches in a Soxhlet extractor with 96 L of 95% ethanol per batch, for successive periods of 6, 15, and 24 h. The combined ethanol extracts were concentrated in vacuo and partitioned between water (15 L) and ethyl acetate (four 12-L portions). Concentration of the ethyl acetate layer gave a residue which was partitioned between 10% aqueous methanol (12 L) and petroleum ether (three 12-L portions). The aqueous methanol-soluble material was taken up in 3 L of methanol–ethyl acetate (1:4) and filtered through a column of alumina (4.5 kg; activity II–III). The alumina was washed with an additional 6 L of methanol–ethyl acetate, and the combined filtrates were evaporated to give a residue which was subjected to column chromatography on alumina (6.5 kg, activity II–III) with ether followed by ether containing increasing amounts of methanol as eluent. Elution with 10% methanol–ether gave fraction A (10 g), and elution with methanol gave fraction B (48 g).

Isolation of Baccharin (1). Fraction A was further fractionated by column chromatography on silica gel 60 (1 kg). Elution with 2% methanol–chloroform gave fraction C (3 g) which was crystallized from methanol–chloroform. Recrystallization from acetone–hexane gave baccharin (**1**, 1.1 g, 0.002%): mp 238–240 °C; $[\alpha]^{24}_D$ +41.5° (*c* 2.2, $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 259 nm (18 700); IR ($CHCl_3$) 3600, 3450, 1760, 1720, 1650, 1605 cm^{-1}; NMR ($CDCl_3$) δ 0.75 (3 H, s, 14-H), 1.20 (3 H, d, J = 5.6 Hz, 14′-H), 1.37 (3 H, s, 16-H), 1.65 (3 H, s, 12′-H), 2.48 (1 H, dd, J = 16 and 8.8 Hz, 3α-H), 2.75, 3.16 (each 1 H, d, J = 4 Hz, 13-H), 3.11 (1 H, d, J = 5.8 Hz, 10-H), 3.37 (1 H, s, 2′-H), 4.24, 4.42 (2 H, AB q, J = 12.2 Hz, 15-H), 5.8 (1 H, m, 4-H), 5.82 (1 H, d, J = 11 Hz, 10′-H), 5.98 (1 H, dd, J = 15.5 and 2 Hz, 7′-H), 6.60 (1 H, dd, J = 11 and 11 Hz, 9′-H), 7.48 (1 H, dd, J = 15.5 and 11 Hz, 8′-H); mass spectrum (chemical ionization: methane reagent gas) *m/e* 563.2476 (M^+ + H, calcd for $C_{29}H_{39}O_{11}$, 563.2492).

Anal. Calcd for $C_{29}H_{38}O_{11}$: C, 61.91; H, 6.81. Found: C, 61.78; H, 6.81.

Isolation of Isobaccharin (4). Continued elution of the fraction A column with 2% methanol in chloroform gave fraction D (0.37 g). Fraction D was purified by preparative TLC, first on silica gel with 8% methanol in chloroform as eluent, then on alumina with 25% 2-propanol in benzene as eluent, to give a residue which was crystallized from methanol–chloroform. Recrystallization from acetone–hexane gave isobaccharin (**4**, 0.10 g, 0.00018%): mp 228–230 °C; $[\alpha]^{24}_D$ +42° (*c* 0.36, $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 260 nm (21 300); IR (KBr) 3470, 1755, 1710, 1650, 1605 cm^{-1}; NMR ($CDCl_3$) δ 0.76 (3 H, s, 14-H), 1.17 (3 H, d, J = 6.6 Hz, 14′-H), 1.34 (3 H, s, 16-H), 1.68 (3 H, s, 12′-H), 2.47 (1 H, dd, J = 16 and 8 Hz, 3α-H), 2.75, 3.16 (each 1 H, d, J = 4 Hz, 13-H), 3.09 (1 H, d, J = 6 Hz, 10-H), 3.35 (1 H, s, 2′-H), 4.22, 4.47 (2 H, AB q, J = 12.2 Hz, 15-H), 5.8 (1 H, m, 4-H), 5.80 (1 H, d, J = 11 Hz, 10′-H), 5.93 (1 H, dd, J = 16 and 3 Hz, 7′-H), 6.60 (1 H, dd, J = 11 and 11 Hz, 9′-H), 7.44 (1 H, dd, J = 16 and 11 Hz, 8′-H); mass spectrum (chemical ionization: methane reagent gas) *m/e* 563.2493 (M^+ + H, calcd for $C_{29}H_{39}O_{11}$, 563.2492).

Anal. Calcd for $C_{29}H_{38}O_{11}\cdot H_2O$: C, 59.99; H, 6.94. Found: C, 59.79; H, 6.94.

Isolation of Baccharinol (2). Fraction B was subjected to column chromography on silica gel 60 (1 kg). Elution with 10% methanol in ether yielded fractions E and F. Fraction E was combined with similar material obtained from column chromatography or preparative TLC of adjacent fractions and crystallized from methanol–chloroform. Recrystallization from acetone–hexane gave baccharinol (**2**, 3.5 g, 0.0065%): mp 259–263 °C from methanol–chloroform–ether; $[\alpha]^{24}_D$ +165° (*c* 0.50, MeOH); UV (EtOH) λ_{max} (ϵ) 260 nm (20 400); IR (KBr) 3360, 1750, 1715, 1640, 1600 cm^{-1}; NMR ($CDCl_3$) δ 0.83 (3 H, s, 14-H), 1.18 (3 H, d, J = 6 Hz, 14′-H), 1.59 (3 H, s, 12′-H), 1.83 (3 H, s, 16-H), 2.50 (1 H, dd, J = 15 and 8 Hz, 3α-H), 2.88, 3.13 (each 1 H, d, J = 4 Hz, 13-H), 3.44 (1 H, s, 2′-H), 4.24, 4.44 (2 H, AB q, J = 12 Hz, 15-H), 5.46 (1 H, d, J = 5 Hz, 10-H), 5.8 (1 H, m, 4-H), 5.83 (1 H, d, J = 11 Hz, 10′-H), 6.02 (1 H, dd, J = 15 and 3 Hz, 7′-H), 6.63 (1 H, dd, J = 11 and 11 Hz, 9′-H), 7.42 (1 H, dd, J = 15 and 11 Hz, 8′-H); mass spectrum (chemical ionization: methane reagent gas) *m/e* 563.2465 (M^+ + H, calcd for $C_{29}H_{39}O_{11}$, 563.2492).

Anal. Calcd for $C_{29}H_{38}O_{11}$: C, 61.91; H, 6.81. Found: C, 61.69; H, 6.87.

Isolation of Isobaccharinol (3). Preparative TLC of fraction F on silica gel with 10% methanol in chloroform as eluent gave baccharin (**2**) which was combined with fraction E and a residue which was crystallized from methanol–chloroform–ether. Recrystallization from acetone–hexane gave isobaccharinol (**3**, 0.20 g, 0.00037%): mp 249–251 °C; $[\alpha]^{24}_D$ + 149° (*c* 0.66, MeOH); UV (EtOH) λ_{max} (ϵ) 260 nm (20 400); IR (KBr) 3420, 1750, 1720, 1650, 1605 cm^{-1}; NMR ($CDCl_3$) δ 0.83 (3 H, s, 14-H), 1.16 (3 H, d, J = 6 Hz, 14′-H), 1.65 (3 H, s, 12′-H), 1.83 (3 H, s, 16-H), 2.84, 3.13 (each 1 H, d, J = 4 Hz, 13-H), 3.38 (1 H, s, 2′-H), 4.24, 4.46 (2 H, AB q, J = 12 Hz, 15-H), 5.52 (1 H, d, J = 5 Hz, 10-H), 5.8 (1 H, m, 4-H), 5.81 (1 H, d, J = 11 Hz, 10′-H), 5.92 (1 H, dd, J = 15 and 3 Hz, 7′-H), 6.59 (1 H, dd, J = 11 and 11 Hz, 9′-H), 7.40 (1 H, dd, J = 15 and 11 Hz, 8′-H); mass spectrum (chemical ionization: methane reagent gas) *m/e* 563.2493 (M^+ + H, calcd for $C_{29}H_{39}O_{11}$, 563.2492).

Anal. Calcd for $C_{29}H_{38}O_{11}$: C, 61.91; H, 6.81. Found: C, 61.86; H, 6.85.

Baccharinol Triacetate (7). A solution of 50 mg of baccharinol (**2**) in 2 mL of pyridine and 1 mL of acetic anhydride was allowed to stand at room temperature for 18 h. The solvent was removed in vacuo and the residue was crystallized from dichloromethane–hexane to give 47 mg of baccharinol triacetate (**7**): mp 255–257 °C; $[\alpha]^{28}_D$ +145° (*c* 0.39, $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 257 nm (18 700); IR (KBr) 1750 (sh), 1735, 1715 (sh), 1640, 1600 cm^{-1}; NMR ($CDCl_3$) δ 0.80 (3 H, s, 14-H), 1.22 (3 H, d, J = 6.3 Hz, 14′-H), 1.71 (6 H, s, 16-H and 12′-H), 2.03, 2.09, 2.16 (each 3 H, s, -OAc), 2.81, 3.13 (each 1 H, d, J = 4 Hz, 13-H), 3.50 (1 H, s, 2′-H), 4.29, 4.53 (2 H, AB q, J = 12 Hz, 15-H), 5.57 (1 H, d, J = 5 Hz, 10-H), 5.8 (1 H, m, 4-H), 5.81 (1 H, d, J = 11 Hz, 10′-H), 5.90 (1 H, dd, J = 15.5 and 3 Hz, 7′-H), 6.60 (1 H, dd, J = 11 and 11 Hz, 9′-H), 7.41 (1 H, dd, J = 15.5 and 11 Hz, 8′-H); mass spectrum (chemical ionization: methane reagent gas) *m/e* 689.2830 (M^+ + H, calcd for $C_{35}H_{45}O_{14}$, 689.2809).

Anal. Calcd for $C_{35}H_{44}O_{14}$: C, 61.04; H, 6.44. Found: C, 61.21; H, 6.42.

8-Ketobaccharinol (8). Baccharinol (**2**, 56 mg, 0.1 mmol), pyridinium chlorochromate (34 mg, 0.15 mmol), and anhydrous sodium acetate (3 mg, 0.3 mmol) were stirred in 4 mL of dichloromethane for 1.5 h. The reaction mixture was filtered, the solids were washed with 10 mL of additional dichloromethane, and the combined filtrate was evaporated in vacuo. Preparative TLC on silica gel with 10% methanol in chloroform as eluent followed by crystallization from 1,2-dichloroethane–ether yielded 8-ketobaccharinol (**8**, 27 mg): mp 265–266 °C; $[\alpha]^{26}_D$ +127° (*c* 0.39, $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 259 nm (19 300), 235 (sh) (14 300); IR (KBr) 3450, 1760, 1715, 1680, 1645, 1605 cm^{-1}; NMR ($CDCl_3$) δ 0.81 (3 H, s, 14-H), 1.19 (3 H, d, J = 5.5 Hz, 14′-H), 1.49 (3 H, s, 12′-H), 1.85 (3 H, s, 16-H), 2.86, 3.16 (each 1 H, d, J = 4 Hz, 13-H), 3.53 (1 H, s, 2′-H), 4.15, 4.45 (2 H, AB q, J = 12.5 Hz, 15-H), 5.8 (1 H, m, 4-H), 5.85 (1 H, d, J = 11 Hz, 10′-H), 6.05 (1 H, dd, J = 15.5 and 3 Hz, 7′-H), 6.62 (1 H, dd, J = 11 and 11 Hz, 9′-H), 6.63 (1 H, br d, J = 5 Hz, 10-H), 7.52 (1 H, dd, J = 15.5 and 11 Hz, 8′-H); mass spectrum (chemical ionization: methane reagent gas) *m/e* 561.2320 (M^+ + H, calcd for $C_{29}H_{37}O_{11}$, 561.2336).

Anal. Calcd for $C_{29}H_{36}O_{11}$: C, 62.13; H, 6.47. Found: C, 61.89; H, 6.49.

Hydrolysis of Baccharin (1). A solution of 25 mg of baccharin (1) and 20 mg of lithium hydroxide monohydrate in methanol was stirred at room temperature for 3 h. The reaction mixture was passed through a small column (ca. 5 g) of Dowex 50W-X8 ion-exchange resin and the methanol was evaporated. The residue was treated with excess ethereal diazomethane for 30 min. Evaporation of the solvent followed by preparative TLC on silica gel, developed with 6% methanol in chloroform, gave trichothecene **6** (9.2 mg), a compound previously described,[8] and dimethyl ester **5** (7.9 mg), as a colorless glass: $[\alpha]^{19}_D$ +60° (*c* 1.19, $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 256 nm (20 000); IR ($CHCl_3$) 3500, 1745, 1710, 1640, 1610 cm^{-1}; NMR ($CDCl_3$) δ 1.14 (3 H, d, J = 6 Hz, 14-H), 1.37 (3 H, s, 12-H), 3.74, 3.81 (each 3 H, s,

–$COOCH_3$), 5.74 (1 H, d, J = 11 Hz, 10′-H), 5.83 (1 H, dd, J = 15 and 7 Hz, 7′-H), 6.59 (1 H, dd, J = 11 and 11 Hz, 9′-H), 7.59 (1 H, dd, J = 15 and 11 Hz, 8′-H); mass spectrum m/e 300, 285, 268, 211, 193, 187, 161, 159, 141, 137, 109.

Methanolysis of Baccharinol (2). To a solution of 25 mg of baccharinol (**2**) in 2 mL of dry methanol at room temperature under argon was added slowly 0.4 mL of 1.4 M n-butyllithium in hexane. The mixture was stirred for 2 h, poured onto a small column of silica gel, and washed with 20% methanol in ethyl acetate. The filtrate was evaporated and the residue subjected to preparative TLC on silica gel, developed with 10% methanol in chloroform, to give dimethyl ester **5** (9.7 mg) identical to that obtained from baccharin (**1**) and the trihydroxytrichothecene **9** (11.1 mg), which was crystallized from acetone–hexane: mp 185–186 °C; $[\alpha]^{20}_D$ + 14.5° (c 0.38, MeOH); NMR ($CDCl_3$–CD_3OD, 9:1) δ 0.80 (3 H, s, 14-H), 1.70 (3 H, s, 16-H), 2.38 (1 H, dd, J = 16 and 8 Hz, 3α-H), 2.73, 2.97 (each 1 H, d, J = 4 Hz, 13-H), 3.69 (2 H, s, 15-H), 3.96 (1 H, dd, J = 8 and 4 Hz, 8-H), 4.49 (1 H, dd, J = 8 and 3.4 Hz, 4-H), 5.32 (1 H, d, J = 5.4 Hz, 10-H); mass spectrum m/e 282.14659 (M^+, calcd for $C_{15}H_{22}O_5$, 282.14671).

Anal. Calcd for $C_{15}H_{22}O_5$: C, 63.81; H, 7.86. Found: C, 63.95; H, 7.82.

Acetylation of 4β,8β,15-Trihydroxy-12,13-epoxytrichothecene (9). A solution of 29 mg of **9** in 1 mL each of pyridine and acetic anhydride was allowed to stand at room temperature for 18 h. The solvent was removed in vacuo and the residue crystallized from benzene–hexane to give **10**, 20 mg: mp 124–126 °C; $[\alpha]_D$ +26° (c 0.55, $CHCl_3$); IR ($CHCl_3$) 1735 cm^{-1}; NMR ($CDCl_3$) δ 0.80 (3 H, s, 14-H), 1.70 (3 H, s, 16-H), 2.0 (3 H, m, 3β-H, 7-H), 2.07, 2.08, 2.11 (each 3 H, s, –OAc), 2.52 (1 H, dd, J = 15.5 and 7.5 Hz, 3α-H), 2.85 (1 H, d, J = 5.1 Hz, 2-H), 2.82, 3.14 (each 1 H, d, J = 4 Hz, 13-H), 3.70 (1 H, d, J = 5.8 Hz, 11-H), 4.06, 4.23 (2 H, AB q, J = 12.5 Hz, 15-H), 5.24 (1 H, dd, J = 8 and 4 Hz, 8-H), 5.6 (2 H, m, 4-H and 10-H); mass spectrum m/e 408.17939 (M^+, calcd for $C_{21}H_{28}O_8$, 408.17840).

Anal. Calcd for $C_{21}H_{28}O_8$: C, 61.75; H, 6.91. Found: C, 61.66; H, 7.02.

Methanolysis of Isobaccharinol (3). By a procedure similar to that described for **2**, isobaccharinol gave the trihydroxytrichothecene **9**, identical to that obtained from **2**, and the dimethyl ester **11**: $[\alpha]^{27}_D$ +38° (c 0.14, $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 257 nm (19 100); IR ($CHCl_3$) 3500, 1750, 1720, 1645, 1610 cm^{-1}; NMR ($CDCl_3$) δ 1.13 (3 H, d, J = 6 Hz, 14-H), 1.36 (3 H, s, 12-H), 3.74, 3.81 (each 3 H, s, –$COOCH_3$), 5.73 (1 H, d, J = 11 Hz, 10′-H), 5.96 (1 H, dd, J = 15.5 and 8 Hz, 7′-H), 6.60 (1 H, dd, J = 11 and 11 Hz, 9′-H), 7.56 (1 H, dd, J = 15.5 and 11 Hz, 8′-H); mass spectrum m/e 300, 285, 268, 211, 193, 187, 161, 159, 141, 137, 109.

Methanolysis of Isobaccharin (4). By a procedure similar to that described for **2**, isobaccharin (**4**) gave **6** and **11**, identical to those described above.

Catalytic Hydrogenation of Baccharinol (2). A solution of baccharinol (**2**, 25 mg) in 25 mL of absolute ethanol was hydrogenated at atmospheric pressure using 10% palladium on charcoal (9 mg) as catalyst. After 2 equiv of hydrogen was taken up, the catalyst was removed by filtration and the solvent evaporated to afford a colorless glass. Crystallization from acetone–hexane gave tetrahydrobaccharinol (**14**): mp 245–246 °C; $[\alpha]^{22}_D$ +32° (c 0.53, $CHCl_3$); IR (KBr) 3430, 1745, 1735 cm^{-1}; NMR ($CDCl_3$) δ 0.83 (3 H, s, 14-H), 1.13 (3 H, d, J = 6 Hz, 14′-H), 1.51 (3 H, s, 12′-H), 1.83 (3 H, s, 16-H), 2.85, 3.16 (each 1 H, d, J = 4 Hz, 13-H), 3.36 (1 H, s, 2′-H), 4.16, 4.31 (2 H, AB q, J = 12 Hz, 15-H), 5.47 (1 H, d, J = 5 Hz, 10-H), 5.70 (1 H, dd, J = 7.5 and 3.5 Hz, 4-H); mass spectrum (chemical ionization: methane reagent gas) 567.2811 (M^+ + H, calcd for $C_{29}H_{43}O_{11}$, 567.2805).

Anal. Calcd for $C_{29}H_{42}O_{11}$: C, 61.47; H, 7.47. Found: C, 61.49; H, 7.48.

Catalytic Hydrogenation of Isobaccharinol (3). By the same procedure as that described above, isobaccharinol (**3**) afforded tetrahydroisobaccharinol (**12**): mp 248–250 °C; $[\alpha]^{22}_D$ +35° (c 0.27, $CHCl_3$); IR (KBr) 3400, 1755, 1735 cm^{-1}; NMR ($CDCl_3$) δ 0.83 (3 H, s, 14-H), 1.15 (3 H, d, J = 6.5 Hz, 14′-H), 1.51 (3 H, s, 12′-H), 1.83 (3 H, s, 16-H), 2.85, 3.16 (each 1 H, d, J = 4 Hz, 13-H), 3.40 (1 H, s, 2′-H), 4.12, 4.38 (2 H, AB q, J = 12 Hz, 15-H), 5.50 (1 H, br d, J = 5 Hz, 10-H), 5.71 (1 H, dd, J = 8 and 4 Hz, 4-H); mass spectrum (chemical ionization: methane reagent gas) 567.2811 (M^+ + H, calcd for $C_{29}H_{43}O_{11}$, 567.2805).

Anal. Calcd for $C_{29}H_{42}O_{11}$: C, 61.47; H, 7.47. Found: C, 61.47; H, 7.50.

Oxidation of Tetrahydrobaccharinol (14). Tetrahydrobaccharinol (**14**, 22 mg, 0.04 mmol), pyridinium chlorochromate (45 mg, 0.21 mmol), and anhydrous sodium acetate (5 mg, 0.06 mmol) were stirred in 2 mL of dichloromethane for 2 h. The reaction was filtered and the solids were washed twice with 2 mL of dichloromethane. The combined filtrates were extracted with 10 mL of water, and the solvent was evaporated. Preparative TLC on silica gel, developed with 6% methanol in chloroform, gave a colorless glass (17 mg). Crystallization from acetone–hexane afforded **13**: mp 260–262 °C; $[\alpha]^{24}_D$ +49° (c 0.34, $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 227 nm (8450); IR (KBr) 3400, 1760, 1730, 1720, 1690 cm^{-1}; NMR δ 0.82 (3 H, s, 14-H), 1.44 (3 H, s, 12′-H), 1.85 (3 H, br s, 16-H), 2.17 (3 H, s, 14′-H), 2.85, 3.17 (each 1 H, d, J = 4 Hz, 13-H), 3.51 (1 H, s, 2′-H), 4.05, 4.47 (2 H, AB q, J = 12.5 Hz, 15-H), 5.80 (1 H, dd, J = 7.5 and 4 Hz, 4-H), 6.52 (1 H, dq, J = 4 and 1.5 Hz, 10-H); mass spectrum (chemical ionization: methane reagent gas) 563.2482 (M^+ + H, calcd for $C_{29}H_{39}O_{11}$, 563.2492).

Anal. Calcd for $C_{29}H_{38}O_{11}$: C, 61.91; H, 6.81. Found: C, 61.67; H, 6.88.

Oxidation of Tetrahydroisobaccharinol (12). By the same procedure as that described for **14**, tetrahydroisobaccharinol (**12**) gave **13**, identical to that obtained from **14**.

Registry No.—**1**, 61251-97-6; **2**, 63783-94-8; **3**, 63814-57-3; **4**, 63814-58-4; **5**, 63783-95-9; **6**, 63783-96-0; **7**, 63783-97-1; **8**, 63783-98-2; **9**, 63783-99-3; **10**, 63784-00-9; **11**, 63814-59-5; **12**, 63784-01-0; **13**, 63784-02-1; **14**, 63814-60-8.

References and Notes

(1) Tumor Inhibitors 125. Part 124: S. M. Kupchan, A. T. Sneden, A. R. Branfman, G. A. Howie, L. I. Rebhun, W. E. McIvor, R. W. Wang, and T. C. Schnaitman, *J. Med. Chem.*, submitted for publication.

(2) This investigation was supported by grants from the National Cancer Institute (CA-11718) and American Cancer Society (CH-42L and CH-42M), and by a contract with the Division of Cancer Treatment, National Cancer Institute (N01-CM-67002). One of us (B.B.J.) wishes to thank the National Institutes of Health for a National Research Service Award (1F32 CA05368-01).

(3) Visiting Scholar, 1975–1976; on sabbatical leave, University of Maryland, College Park, Md.

(4) Leaves, twigs, and flowers were collected in Brazil in May 1975. The authors acknowledge with thanks receipt of the dried plant material from Dr. R. E. Perdue, Jr., United States Department of Agriculture, Baltimore, Md., in accordance with the program developed by the National Cancer Institute.

(5) Antileukemic activity was assayed under the auspices of the National Cancer Institute by the procedures described by R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3**, 1 (1972).

(6) S. Morris Kupchan, Bruce B. Jarvis, Richard G. Dailey, Jr., William Bright, Robert F. Bryan, and Yoshikazu Shizuri, *J. Am. Chem. Soc.*, **98**, 7092 (1976).

(7) Baccharin (**1**) and isobaccharin (**4**) gave typical *T/C* values in the range of 180–230 at dose levels of 0.5 to 8 mg/kg against PS. Baccharinol (**2**) and isobaccharinol (**3**) showed *T/C* values of 160–220 in the dosage range of 0.5 to 4 mg/kg. All four compounds showed cytotoxicity (ED_{50}) against KB cell culture at 10^{-3}–10^{-4} μg/mL.

(8) W. Zürcher, J. Gutzwiller, and Ch. Tamm, *Helv. Chim. Acta*, **48**, 840 (1965).

(9) J. R. Bamburg, N. V. Riggs, and F. M. Strong, *Tetrahedron*, **24**, 3329 (1968).

(10) A single crystal x-ray analysis of a related compound from *B. megapotamica* proves conclusively the β stereochemistry of the C-8 hydroxyl. Details will be presented in a forthcoming publication.

(11) (a) W. Breitenstein and Ch. Tamm, *Helv. Chim. Acta*, **58**, 1172 (1975). (b) J. R. Hanson, T. Marten, and M. Siverns, *J. Chem. Soc., Perkin Trans. 1*, 1033 (1974). (c) R. A. Ellison and F. N. Kotsonis, *J. Org. Chem.*, **41**, 576 (1976).

(12) J. R. Bamburg and F. M. Strong, *Microbial Toxins*, **7**, 207 (1971).

(13) J. R. Bamburg, *Adv. Chem. Ser.*, **149**, 144 (1976).

(14) Ch. Tamm, *Fortschr. Chem. Org. Naturst.*, **31**, 63 (1974).

(15) B. Böhner and Ch. Tamm, *Helv. Chim. Acta*, **49**, 2547 (1966). Roridin D was also isolated from *B. megapotamica* and found to be inactive against the P-388 leukemia in mice.

(16) Deceased Oct. 19, 1976.