Isolation of Macrocyclic Trichothecenes from a Large-Scale Extract of *Baccharis megapotamica*

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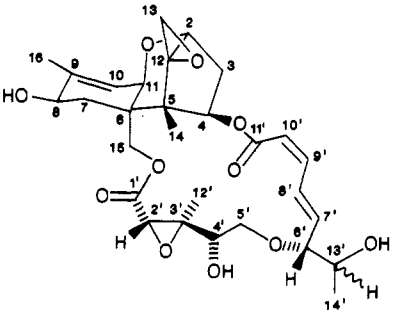
The isolation and characterization of 13 new macrocyclic trichothecenes from the Brazilian plant *Baccharis megapotamica* are reported. A large-scale extraction of 1800 kg of plant material yielded a large number of new macrocyclic trichothecenes, most of which occurred as sets of diastereomers, epimeric at C13′. Structural novelties observed in some of these toxins include hydroxylation at C16 and the presence of an α,β-unsaturated ketone in the A ring.

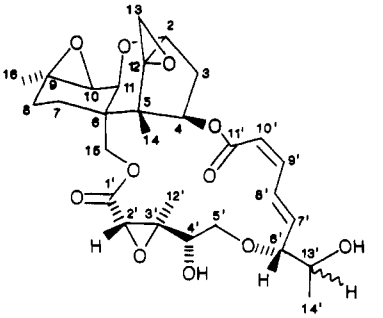
An extract of the Brazilian plant *Baccharis megapotamica* was shown to contain a series of compounds that exhibited high activity in vivo against P388 leukemia in mice and high cytotoxicity in vitro against KB cells.[1] These compounds, called baccharinoids, belong to the macrocyclic trichothecene antibiotic complex,[2] which includes the verrucarins and roridins; the baccharinoids are closely related in structure to the roridins. Up to this finding, trichothecenes have been found to be produced only by various soil fungi, e.g., *Fusarium*, *Trichothecium*, *Trichoderma*, *Myrothecium*, *Cephalosporium*, *Stachybotrys*, *Verticimonosporium*, and *Cylindrocarpon*,[3] although recently there is a report of the isolation of roridins A and E and the closely related miotoxins from another Brazilian *Baccharis* species, *B. coridifolia*.[4] In addition, the presence of trichothecenes in these Brazilian *Baccharis* plants is unexpected, since trichothecenes are known to be potent phytotoxic agents.[5] The principal difference in structure between the baccharinoids and the roridins is the presence of an extra oxygen atom, in the form of either an 8β-hydroxy group or a 9β,10β-epoxy group, in the A ring of the baccharinoids. It is believed that these *Baccharis* species acquire roridins from a fungal source, and, in the case of *B. megapotamica*, the plant oxidizes the roridins to baccharinoids.[6]

Earlier work in the laboratory of the late Prof. Morris Kupchan established that there were eight principal baccharinoids in *B. metapotamica*; however, TLC and HPLC analyses of the chromatography fractions of these earlier extracts suggested the presence of many additional compounds of similar structure. Details about the isolation and characterization of four of the principal baccharinoids (B4, B5, B6, and B8) have been reported,[1] and the details about the other four major congeners (B1, B2, B3, and B7) will be reported elsewhere.

Initially, these macrocyclic trichothecenes were referred to as baccharinoids B1, B2, B3, etc., based on the chronology of their isolation. However, it soon became evident that the baccharinoids were present as sets of diastereomers, epimeric at C13′, e.g., baccharinoids B4 (1) and B6 (2) and B5 (3) and B8 (4). Thus, starting with baccharinoids B9 and B10, the naming of these compounds is such that the epimers appear in sequence; however, in some cases, we were unable to isolate one of the epimers in the set.



B4 (1), C13′ *R*
B6 (2), C13′ *S*



B5 (3), C13′ *R*
B8 (4), C13′ *S*

In order to acquire sufficient material for further testing, a large-scale collection (ca. 1800 kg) of *B. megapotamica* was made in 1978, but the dried plant material was not extracted until 1981. Herein, we describe the isolation of a series of trichothecenes obtained from the 2-propanol extract of this large-scale collection.

Results and Discussion

The ground, dried plant material (ca. 1800 kg) was extracted at 50–55 °C with 6000 gal of 2-propanol. The

(1) Kupchan, S. M.; Streelman, D. R.; Jarvis, B. B.; Dailey, R. G., Jr.; Sneden, A. T. *J. Org. Chem.* **1977**, *42*, 4221.
(2) (a) Tamm, C. *Fortschr. Chem. Org. Naturst.* **1974**, *31*, 61. (b) Jarvis, B. B.; Mazzola, E. P. *Acc. Chem. Res.* **1982**, *14*, 388.
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(5) Cutler, H. G.; Jarvis, B. B. *Environ. Exp. Bot.* **1985**, *25*, 115.
(6) Jarvis, B. B.; Midiwo, J. O.; Tuthill, D.; Bean, G. A. *Science (Washington, D.C.)* **1981**, *214*, 460.

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Table I. ^{13}C NMR Spectral Data for Baccharinoids in $CDCl_3$

position	B9 (14)	B10 (15)	B12 (8)	B13 (20)	B14 (21)	B16 (26)	B17 (7)	B20 (16)	B21 (11)	B23 (22)	B24 (23)	B25 (24)	B27 (25)
2	78.8	78.7	79.4	79.3	79.3	79.4	78.8	78.6	79.2	79.2	79.3	78.3	79.4
3	35.1	34.9	75.5	35.6	35.6	35.4	34.8	34.6	35.1	34.9	35.0	35.4	36.1
4	74.1[a]	74.2[a]	83.0	73.9[a]	74.0[a]	74.5[a]	74.3	74.4	74.0	74.5	74.7	75.1	73.6[a]
5	47.8	48.4	49.2	48.7	48.6	48.9	49.1	48.8	49.4	49.2	49.4	48.7	48.3
6	42.5	42.5	44.1	45.2	45.2	43.5	42.9	42.9	45.2	43.9	44.0	45.4	46.5
7	18.3	18.2	20.0	31.7	31.5	20.8	17.6	17.6	30.7	20.1	20.2	31.6	38.8
8	26.3	25.2	27.5	68.1[b]	68.1[b]	23.2	26.4	26.4	67.0[b]	23.2	23.3	68.3[a]	197.1
9	57.5	57.4	139.0	142.4	142.4	143.7	57.7	57.4	142.8	143.6	143.6	142.4	136.6
10	57.5	57.5	119.1	118.0[c]	117.7[c]	117.6[b]	58.2	57.8	120.8	118.3	118.5	121.2[b]	138.5[b]
11	67.7	67.7	67.4	67.1[b]	67.1[b]	67.1	67.5	67.6	68.0[b]	66.8	66.9	66.9[a]	66.4
12	65.0	65.0	64.4	65.5	65.5	65.7	64.9	64.8	65.2	65.3	65.3	65.4	65.7
13	48.5	47.6	46.3	48.0	48.0	48.1	47.6	47.6	47.8	47.9	47.9	48.1	47.9
14	6.9	6.9	6.8	6.8	6.8	6.9	7.1	6.9	7.0	7.0	7.0	6.8	6.4
15	63.1	62.9	63.7	64.2	64.1	65.7[c]	63.7	63.7	65.0	66.0	66.1	64.2	64.6
16	22.3	22.2	23.0	18.6[d]	17.1[d]	63.5[c]	22.2	22.1	18.8	63.5	63.4	18.9	17.4
1′	166.4	166.3	167.9	166.3	166.3	166.8	168.2	172.7	168.1	172.8	172.8	165.8[c]	166.4
2′	115.0	115.0	57.8	121.2[c]	121.1[c]	118.6[b]	57.4	38.3	58.2	38.2	38.4	116.8	114.6[c]
3′	161.0	161.3	63.1	160.5	161.2	161.4	63.7	32.5	63.4	32.4	32.6	158.3[b]	161.1
4′	74.8[a]	75.1[a]	39.8	74.3[a]	74.8[a]	75.1[a]	40.1	73.4	39.7	73.0	73.2	74.3	74.6[a]
5′	74.7	74.3	67.9	73.6	73.1	74.0	67.9	74.2	67.6	74.3	74.7	63.7	71.8
6′	84.2	83.0	84.7	84.0	82.7	83.2	86.0	84.4	85.8	86.0	84.7	165.1[c]	82.3
7′	138.0	137.9	140.6	137.6	137.3	138.1	138.6	139.6	138.5	139.8	139.3	126.5[d]	137.2[b]
8′	126.6	126.6	125.2	126.8	126.9	126.8	126.3	126.3	126.3	126.5	126.8	139.1[e]	127.3
9′	143.7	143.5	143.7	143.5	143.5	143.5	143.5	144.1	142.8	144.0	144.0	139.7[e]	143.4
10′	117.8	117.5	116.7	115.0[c]	114.9[c]	115.0[b]	117.9	117.1	118.0	117.6	117.5	125.9[d]	117.7[c]
11′	166.0	166.2	166.2	165.9	166.3	165.9	166.5	166.7	166.4	166.8	166.8	165.8[c]	165.8
12′	15.7	15.5	17.2	18.9[d]	19.0[d]	18.9	17.4	14.9	17.4	15.0	14.8	13.9	15.5
13′	70.9	68.9	69.1	70.8	68.5	70.8	70.8	69.8	70.9	71.0	70.1		68.2
14′	18.6	17.1	17.9	16.0	15.9	16.0	18.3	17.9	18.3	18.6	18.0		16.1

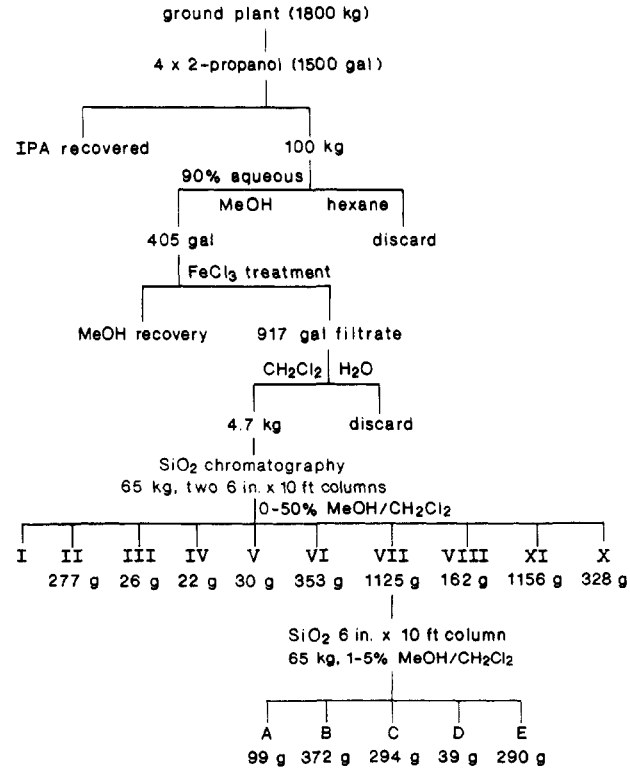
[a-e] Assignments may be reversed.

2-propanol extract stood in cold storage (5 °C) for 18 months before the extract was concentrated and solvent partitioned. It was clear that the procedure used for the original isolation work from 54 kg of *B. megapotamica*[1] would be very cumbersome and expensive because of the numerous chromatographies involved. We sought to develop methods, in addition to the normal solvent partitions, that would decrease the weight of the active crude fraction prior to the first chromatography. The most effective procedure found was the ferric gel treatment, which is a procedure used to clean up environmental samples prior to HPLC or GC analysis.[7] To our knowledge, this procedure has never been applied on a large scale. In a preliminary study, it was shown that a crude extract sample weighing ca. 25 g could be cut to about 5 g in weight by first partitioning against 90% aqueous methanol/hexane followed by ferric gel treatment of the aqueous methanol fraction (analogous to Scheme I). HPLC analysis before and after this procedure indicated that less than 10% of the desired macrocyclic trichothecenes was lost while the weight of the crude extract was cut by 80%. Although this procedure is straightforward and clearly works well on a preparative scale, applying this procedure to a very large plant extract was another matter altogether (see Experimental Section). In the event, the application of Scheme I resulted in a reduction in weight of the crude extract of over 90%. Unfortunately, we estimate that we lost perhaps 50% of the desired compounds.

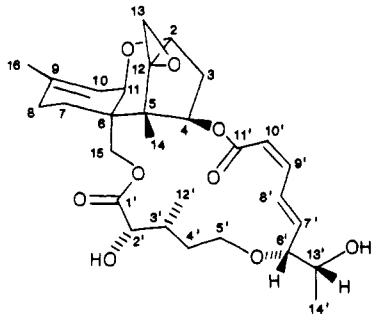
The first silica gel chromatography yielded 10 fractions (Scheme I). Examination of fractions I–V by various chromatographic methods gave no indication of the presence of trichothecenes although we did isolate 2,5-dimethoxybenzoquinone from fraction II and 7-hydroxy-6-methoxycoumarin from fractions III–V.[8]

(7) Scott, P. M. *J. Assoc. Off. Anal. Chem.* **1982**, *65*, 876.
(8) Jarvis, B. B.; Pena, N. B.; Çömezoğlu, S. N.; Rao, M. M. *Phytochemistry* **1986**, *25*, 533.

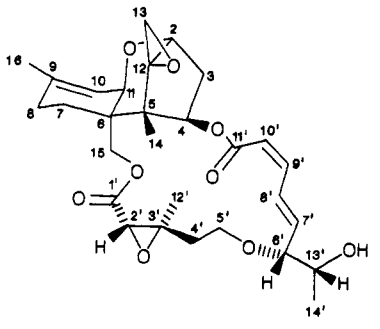
Scheme I. Large-Scale Extract of *B. megapotamica*



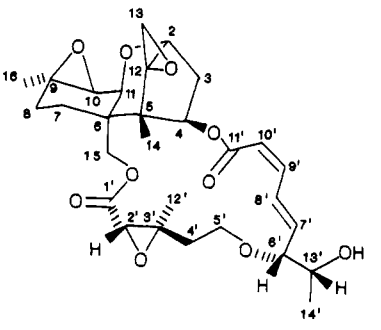
Fraction VI was taken up in dichloromethane and carefully washed with 2.5% aqueous sodium hydroxide. A series of chromatographies of the dichloromethane fraction gave roridins A (5) and D (6) and a new baccharinoid, B17 (7). High-resolution mass spectroscopy (HRMS) of 7 indicated a molecular formula of $C_{29}H_{38}O_{10}$, and ^1H and ^{13}C NMR spectroscopy indicated that the C9,C10 double bond in 7 was epoxidized (see Table I).



roridin A (5)



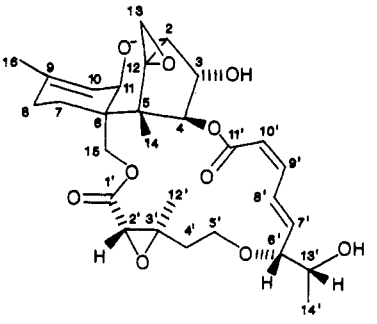
roridin D (6)



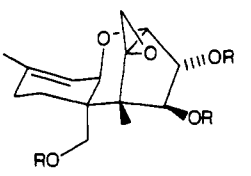
B17 (7)

Epoxidation (*m*-chloroperoxybenzoic acid, MCPBA) of roridin D (6) gave baccharinoid B17 (7). The absolute stereochemistry of roridin D and baccharinoid B17 was established by relating these compounds to roridin E, which possesses the *R* configuration at both C6′ and C13′,[9] and verrucarin B, which possesses the 2′*S*,3′*R* configuration.[10] Treatment of roridin D with pyridinium dichromate (PDC)[11] gave verrucarin B, and diepoxidation of roridin E with MCPBA gave baccharinoid B17. Thus, both roridin D (6) and baccharonoid B17 (7) are 2′*S*,3′*R*,6′*R*,13′*R* in configuration.

Treatment of fraction VII-B (Scheme I) with 2.5% NaOH followed by a filtration chromatography on alumina with 2-propanol in hexane as eluent yielded a fraction that upon crystallization gave baccharinoid B12 (8). Acetylation of baccharinoid B12 (8) gave a diacetate, and both ^{1}H and ^{13}C NMR spectroscopy of 8 indicated that the central trichothecene ring possessed a 3α-hydroxy group. In particular, the signal for H4, which normally appears at ca. δ 5.80 as a doublet of doublets, is shifted to lower frequency (δ 5.61) and appears as a doublet (J = 3.7 Hz). In the ^{1}H NMR spectra of various trichothecenes that have the 3α-hydroxy group, the 4α proton shifts to lower frequency.[3a] In addition, the ^{13}C NMR spectrum of 8 (Table I) has two signals between 80 and 90 ppm; all other macrocyclic trichothecenes have only one signal (C6′) in this region. The signal at δ 83 in 8 is assigned to C4. Hydrolysis of baccharinoid B12 (8) gave scirpentriol (9), which upon acetylation (Ac_2O/triethylamine/DMAP) gave a triacetate 10 identical to that formed upon acetylation of diacetoxyscirpenol.[12] The similarity of the ^{1}H and ^{13}C NMR spectra of roridin D and baccharinoid B12 suggests that these compounds have the same stereochemistry at C2′, C3′, C6′, and C13′, and this has been confirmed by a single crystal X-ray diffraction analysis.[13]

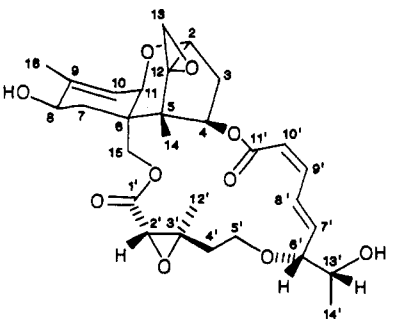


B12 (8)

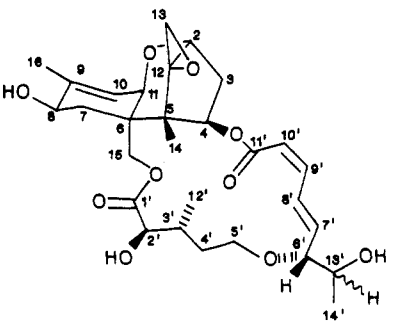


9, R = H
10, R = Ac

Fraction VII-C (Scheme I), which was the principal baccharinoid B5 (3) containing fraction, was subjected to preparative HPLC to give several fractions. Further chromatography yielded baccharinoid B5 (3), megapodiol,[8] and a new trichothecene, baccharinoid B21 (11, 8β-hydroxyroridin D). The ^{1}H NMR spectra of B21 (11) and



B21 (11)



B3 (12), C13′*S*
B7 (13), C13′*R*

roridin D (6) are nearly identical except for the presence of an additional multiplet at δ 3.82–3.89 in the spectrum of 11, which suggests the presence of an extra hydroxy group. The acetylation of 11 gave a diacetate whose ^{1}H NMR spectrum exhibits a one-proton resonance with a five-line pattern at δ 5.02 assignable to H13′. The proton on the carbon bearing the other acetate resonates at δ 5.15

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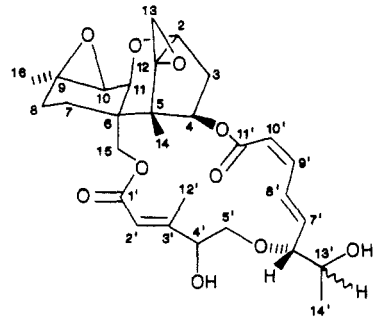
(10) Breitenstein, W.; Tamm, C.; Arnold, E. V.; Clardy, J. *Helv. Chim. Acta* **1979**, *62*, 2699.
(11) Jarvis, B. B.; Midiwo, J. O.; Flippen-Anderson, J. L.; Mazzola, E. P. *J. Nat. Prod.* **1982**, *45*, 440.
(12) Sigg, H. P.; Mauli, R.; Flury, E.; Hauser, D. *Helv. Chim. Acta* **1965**, *48*, 962.
(13) Jarvis, B. B.; Comezoglu, S. N.; Ammon, H. L.; Kerr, C. S., unpublished results.

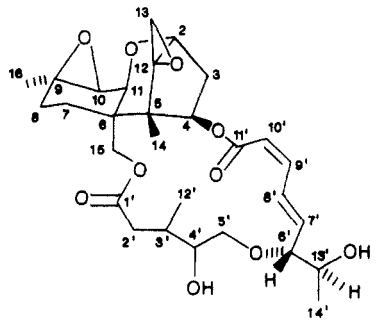
and is a doublet of doublets. A comparison of the ^{13}C NMR spectra of 11 and roridin D (6)[14] shows that the resonances for carbons 7, 8, 9, 10, 11, and 16 differ significantly for these two compounds, which is consistent with the second hydroxy group being 8β. Indeed, hydroxylation of roridin D with SeO_2 gave 8β-hydroxyroridin D, which is identical with baccharinoid B21 (11).

Fraction VII-D (Scheme I) was treated with 2.5% NaOH, and the dichloromethane solubles were triturated with ether. After a filtration chromatography on alumina, the ether-insoluble material gave baccharinoid B5 (3). The ether-soluble material was subjected to filtration chromatography over alumina to give the nontrichothecene sesquiterpene, clovandiol.[8]

A TLC analysis showed that fraction VII-E (Scheme I) contained several trichothecenes, which were isolated following a series of chromatographic separations (Scheme II). Medium-pressure liquid chromatography (MPLC) of fraction E1a (Scheme II) gave fractions containing baccharinoids B5 (3), B8 (4), B3 (12), B7 (13), B9 (14), B10 (15), and B20 (16). Further purification by preparative TLC (PTLC) on either alumina or silica gel yielded these baccharinoids in pure forms.



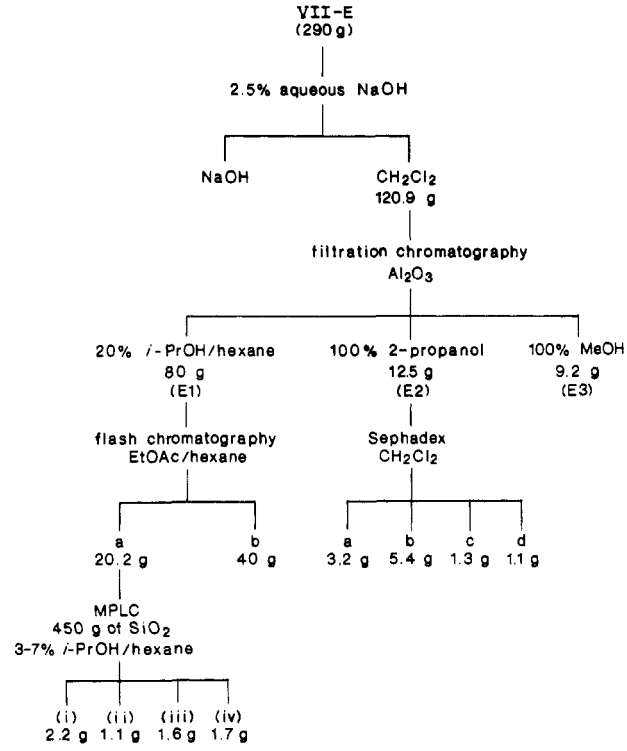
B9 (14), C13'*R*
B10 (15), C13'*S*



B20 (16)

Mass spectrometry established that baccharinoids B9 (14) and B10 (15) were isomeric with the molecular formula $C_{29}H_{38}O_{10}$. The ^1H NMR spectra of baccharinoids B9 and B10 are very similar and resemble in many respects the ^1H NMR spectrum of baccharinoid B5 (3). However, the chemical shifts of protons 12′ and 2′, which are at δ 1.66 and 3.37, respectively, in baccharinoid B5 (3), are at δ 2.31 and 5.98 in 14 and at δ 2.35 and 5.99 in 15. This indicates that the 2′,3′-epoxide in baccharinoid B5 (3) is replaced by a carbon–carbon double bond in 14 and 15. In addition, the ^{13}C NMR spectrum of baccharinoid B5 (3) shows three carbon signals at δ 11.6, 64.4, and 56.0 that correspond to carbons 12′, 3′, and 2′, respectively, while these three signals shift to the high-frequency region, to δ 15.7, 161.0, and 115.0, respectively, in baccharinoid B9 (14) (Table I) and to δ 15.5, 161.3, and 115.0, respectively, in baccharinoid B10 (15) (Table I). Acetylation of 14 and 15 gave diacetates. The H13′ signals appear as a five-line multiplet centered at δ 5.13 in B9 diacetate and as an eight-line multiplet centered at δ 5.00 in B10 diacetate. The second hydroxy group in baccharinoids B9 and B10 was established to be at C4′ as in baccharinoid B5 (3) by comparison of their ^{13}C NMR spectra. In 3, C4′ appears at δ 75.3, and, in baccharinoids B9 (14) and B10 (15), C4′ appears at δ 74.8 and at δ 75.1, respectively.

Scheme II. Fractionation of VII-E



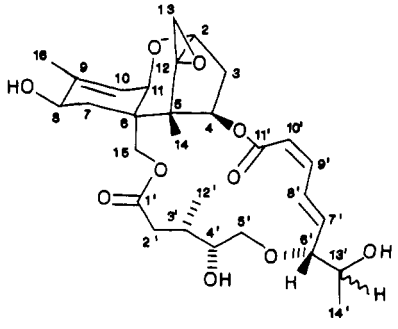
The difference in the multiplicities of these 13′ protons in the ^1H NMR spectra of acetylated baccharinoids B9 and B10 suggests the possibility that the two differ only in stereochemistry at C13′. Further evidence about the site of stereochemical change was obtained from the ^{13}C NMR spectra of these two compounds, which revealed that all the corresponding carbon resonances in the two compounds had nearly identical chemical shifts within a few tenths of a ppm of one another, with only three exceptions: carbons 6′, 13′, and 14′, which appear at δ 84.2, 70.9, and 18.6, respectively, in 14 and at δ 83.0, 68.9, and 17.1 in 15. All the baccharinoids characterized before, as well as most of the roridins, have the *R* configuration at C6′.[11] This suggests that the site of the postulated stereochemical change in baccharinoids B9 and B10 is at C13′. The observation of five-line or eight-line multiplicities centered at ca. δ 5.0 in the acetylated trichothecenes that have an *R* or *S* configuration, respectively, at C13′ suggests that the configuration at C13′ is *R* in baccharinoid B9 (14) and *S* in baccharinoid B10 (15).

Careful analysis of the ^1H and ^{13}C NMR spectra led to the assignment structure of B20 (16). In the ^1H NMR spectrum of 16, the lack of a singlet due to H2′ at ca. δ 5.5 or ca. δ 3.5 suggests that the 2′,3′ region is saturated. The ^1H NMR spectrum of baccharinoid B20 (16) is very similar to that of baccharinoid B1 (17)[13] with a notable exception. The doublet due to H10 at δ 5.50 in 17 is absent in the ^1H NMR spectrum of 16. Instead, a doublet at δ 3.06 appears, indicating that the 9,10 double bond in 17 is replaced by a 9,10-epoxide functionality in 16. This was confirmed by a comparison of the ^{13}C NMR spectra of baccharinoids B1 (17)[13] and B20 (16) (Table I). The principal differences

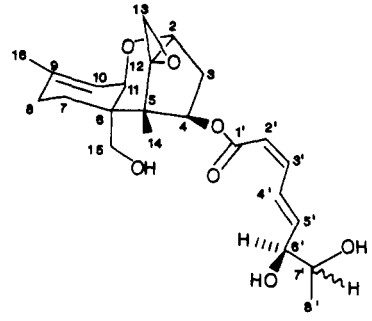
(14) Breitenstein, W.; Tamm, C. *Helv. Chim. Acta* **1975**, *58*, 1172.

are in the chemical shifts of carbons 7, 8, 9, 10, and 16, which are at δ 30.8, 68.2, 142.7, 120.7, and 18.7, respectively, in **17** and at δ 17.6, 26.4, 57.4, 57.8, and 22.1 in **16**. This also suggests the lack of a hydroxy group at C8 in **16**. Upon acetylation, the appearance of two acetate signals at δ 2.06 and 2.11 in the ^{1}H NMR spectrum of **16** indicates the presence of two hydroxy groups. The eight-line multiplet for H13′ in **16** diacetate at δ 4.92 suggests that the configuration at C13′ is *S* on the basis of the previous results. The ^{1}H NMR spectrum of baccharinoid B20 diacetate established the position of the second hydroxy group as being at the C4′ position; H4′ appears as a doublet of doublets at δ 4.83 in B20 diacetate. If the hydroxy group was attached to C2′, it would appear as a doublet, as it does in both baccharinoid B3 diacetate and baccharinoid B7 diacetate (at δ 5.03 and 5.05, respectively).

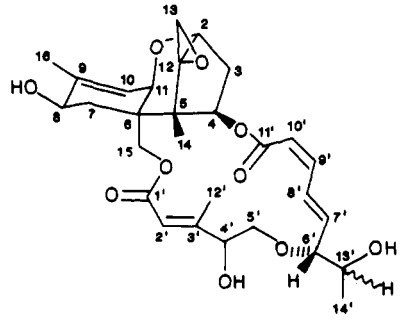
Fraction VIII (Scheme I) was treated with 2.5% NaOH and then subjected to filtration chromatography on alumina to give two fractions. The less polar fraction upon further chromatography yielded baccharinoids B3 (**12**) and B7 (**13**), whose isolation and structural elucidation will be described elsewhere. The more polar fraction was subjected to PTLC on silica gel followed by HPLC separation with an amino-bonded silica gel column to give trichoverrols A and B (**19**).[15]



B1 (17), C13′*R*
B2 (18), C13′*S*



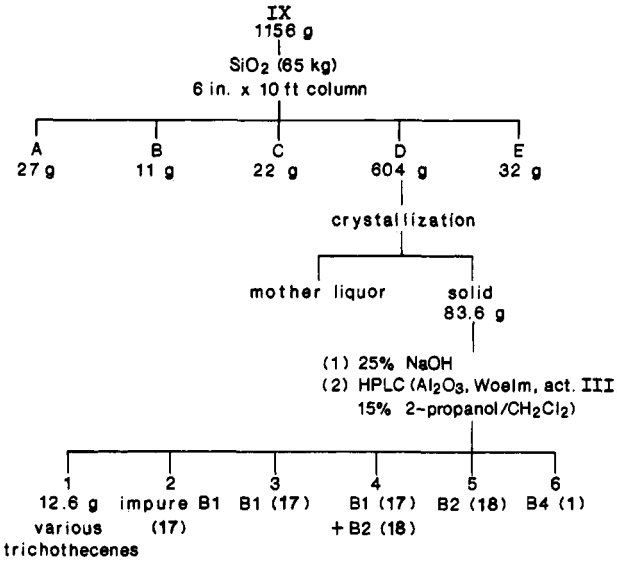
19



B13 (20), C13′*R*
B14 (21), C13′*S*

(15) Jarvis, B. B.; Stahly, G. P.; Pavanasasivam, G.; Midiwo, J. O.; DeSilva, T.; Holmlund, C. E.; Mazzola, E. P.; Geoghegan, R. F., Jr. *J. Org. Chem.* **1982**, *47*, 1117.

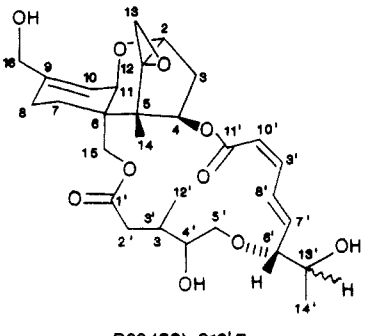
Scheme III. Fraction of IX



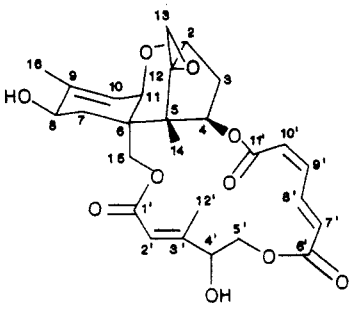
Fraction IX (Scheme I), which contained baccharinoids B1 (**17**), B2 (**18**), and B4 (**1**) in large quantities, was subjected to large-scale silica gel chromatography (Scheme III). Fraction IX-D (Scheme III) was taken up in dichloromethane and precipitated with hexane to yield 84 g of a solid material, which was shown by TLC (Al_2O_3) to be a mixture of baccharinoids B1 (**17**), B2 (**18**), and B4 (**1**). This solid was taken up in dichloromethane, washed with 2.5% aqueous NaOH, and subjected to preparative HPLC on alumina to give 3.1 g of baccharinoid B1 (**17**), 13.6 g of B2 (**18**), and 44 g of B4 (**1**). The mother liquor from the precipitation of fraction IX-D was washed with 2.5% NaOH and subjected to numerous chromatographies to yield additional small quantities of baccharinoids B1, B2, and B4. Fraction IX-D1 was subjected to MPLC (SiO_2, 0–6% methanol in dichloromethane) followed by PTLC and preparative HPLC to give baccharinoids B13 (**20**), B14 (**21**), B23 (**22**), B24 (**23**), B25 (**24**), and B27 (**25**) in pure forms.

The similarities in the ^{1}H NMR and ^{13}C NMR spectra of baccharinoids B13 (**20**) and B14 (**21**) (Table I) and the fact that these compounds have the same molecular formula ($C_{29}H_{38}O_{10}$, as shown by HRMS) suggested the possibility of their being stereoisomers. In their ^{1}H NMR spectra, the appearance of a doublet at ca. δ 5.5 and a singlet at ca. δ 6.2, which correspond to H10 and H2′, respectively, indicates the presence of a double bond at the 9,10- and 2′,3′-positions. Acetylation of both baccharinoids B13 (**20**) and B14 (**21**) gave triacetates in which the protons on the carbon bearing the acetate groups shift to higher frequencies. In B13 triacetate, these signals are centered at δ 5.32, 5.27, and 5.20, and in B14 triacetate, these signals appear at δ 5.33, 5.28, and 4.99. These signals in the acetates can be assigned to H8, H4′, and H13′, respectively. The difference in the multiplicities of H13′ in acetylated B13 and B14 indicates that baccharinoids B13 and B14 differ in stereochemistry at C13′. In B13 triacetate, H13′ gives a five-line multiplet while this signal in B14 triacetate is an eight-line multiplet, again indicating an *R* configuration in baccharinoid B13 (**20**) and an *S* configuration in baccharinoid B14 (**21**) at C13′. Consistent with this assignment are the ^{13}C NMR data for **20** and **21** where only C6′ and C13′ show appreciable differences in their chemical shifts for the two isomers (Table I).

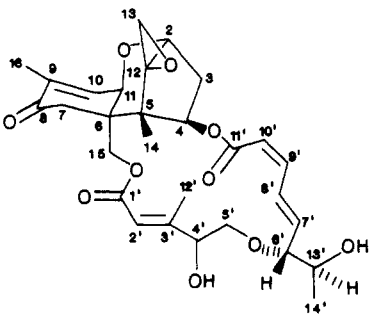
Spectral data (NMR and HRMS) indicate that baccharinoids B23 (**22**) and B24 (**23**) also are epimeric compounds with a molecular formula of $C_{29}H_{40}O_{10}$. However,



B23 (22), C13′*R*
B24 (23), C13′*S*



B25 (24)



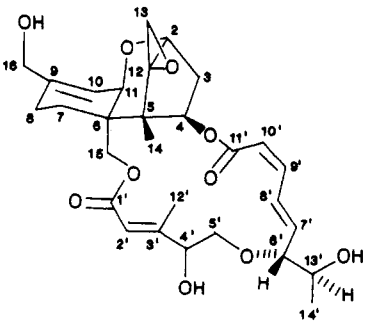
B27 (25)

unlike the other baccharinoids and roridins, the C16 methyl group in **22** and **23** is not found in the normal region (1.5–1.8 ppm) in the ^{1}H NMR spectra. Instead, a two-proton singlet at ca. δ 3.7 appears in the ^{1}H NMR spectra of both compounds, and upon acetylation, these signals shift to δ 4.49. The H10 vinyl proton is present in **22** and **23** but is shifted to a higher frequency, ca. δ 5.7. These data indicate that the C16 methyl group is hydroxylated in baccharinoids B23 (**22**) and B24 (**23**). Several features of the ^{1}H NMR spectra of **22** and **23** and their triacetates were similar with the ^{1}H NMR spectra of baccharinoid B20 (**16**) and its triacetate. Thus, in both **22** and **23** and their triacetates, the H2′ signal appears around δ 2.3. For B23 triacetate, H4′ and H13′ resonate at δ 4.85 (dd) and 5.05 (five-line multiplet), respectively; in B24 triacetate, these signals appear at δ 4.84 (dd) and 4.89 (eight-line multiplet), respectively. These differences in the multiplicities of the 13′ protons in the ^{1}H NMR spectra of acetylated **22** and **23** and the ^{13}C NMR spectral data for **22** and **23** (Table I) establish that the stereochemical change in baccharinoids B23/B24 is at C13′. The chemical shifts of the carbons in **22** and **23** are nearly identical except for carbons 6′ and 13′, which are at δ 86.0 and 71.0, respectively, in baccharinoid B23 (**22**) and at δ 84.7 and 70.1, respectively, in baccharinoid B24 (**23**).

Unlike the other baccharinoids, B25 (**24**) belongs to the verrucarin class of macrocyclic trichothecenes. This is evident from an inspection of the ^{13}C NMR data, which show the presence of only 27 carbon atoms, including three ester carbonyl carbons around δ 165 (Table I). Furthermore, H8′ resonates at δ 8.11, which is at a significantly higher frequency than the chemical shift for H8′ (δ ~7.3) normally observed in roridins and baccharinoids. The full structure of **24** was established principally by NMR spectroscopy. The appearance of a doublet at δ 5.56 (H10) and a singlet at δ 6.07 (H2′) indicates the presence of double bonds at C9,C10 and C2′,C3′. Decoupling experiments on the diacetate of **24** indicated that the hydroxy groups in **24** are located at C8 and C4′. Interestingly, baccharinoid B25 (**24**) and perhaps traces of other verrucarin-type compounds are present in this plant extract in extremely small amounts relative to the roridin-type macrocyclic trichothecenes.

Baccharinoid B27 (**25**) was another uncommon congener isolated from *B. megapotamica*. The ^{1}H and ^{13}C NMR spectra of **25** (Table I) show some differences when compared with the spectra of other baccharinoids. A carbon signal at δ 197 appears in the ^{13}C NMR spectrum and can be assigned to C8, part of an α,β-unsaturated ketone system. Carbons 6, 7, 8, 9, and 10 differ in their chemical shifts when compared with those carbons in trichothecenes containing the 8β-hydroxy/9,10-double-bond system such as baccharinoid B4 (**1**). These carbons in baccharinoid B4 (**1**) are at δ 44.7, 29.6, 66.7, 143.2, and 119.4, respectively, but in baccharinoid B27 (**25**), they occur at 46.5, 38.8, 197.1, 136.6, and 138.5, respectively. In the ^{1}H NMR spectrum of **25**, H10 and H11 appear at ca. δ 6.57 and at ca. δ 4.30, respectively, while in other baccharinoids with a 9,10 double bond, these signals are shifted to δ 5.30 and 3.40, respectively. These data suggest that there is an oxygen functionality in the form of ketone at C8. Furthermore, H7 exhibits a two-proton AB pattern at δ 2.42 and 2.94. The appearance of two singlets at δ 6.18 and 2.24, which are due to H2′ and H12′, respectively, indicates the presence of a double bond at C2′,C3′. Acetylation of baccharinoid B27 (**25**) gave a diacetate whose ^{1}H NMR spectrum, upon decoupling experiments, established that the hydroxy groups in **25** are at C4′ and C13′. Since H13′ is an eight-line multiplet in the ^{1}H NMR spectrum of B27 diacetate, the configuration at C13′ is *S* in baccharinoid B27 (**25**).

Fraction IX-E (Scheme III) was washed with aqueous NaOH and subjected to numerous chromatographies, which gave additional amounts of trichoverrols A and B (**19**) and B16 (**26**). The structure of baccharinoid B16 (**26**)



B16 (26)

was established by ^{1}H NMR and ^{13}C NMR spectra and decoupling experiments. The shift of the C16 protons to δ 3.98 indicates the presence of a hydroxy group at C16. This was confirmed by acetylation, which caused these protons at C16 to shift to higher frequency at δ 4.45. The positions of the two additional hydroxy groups in **26** were shown to be at C4′ and C13′ by NMR spectroscopy (see Table I). The eight-line multiplet for H13′ at δ 5.20 in B16 diacetate establishes the *S* configuration at C13′ in **26**.

Fraction X upon additional fractionation yielded no

additional baccharinoids. Other fractions not specifically discussed in the Experimental Section gave only previously characterized baccharinoids (e.g., baccharinoids B1, B2, B3, and B7, which are to be the subject of another publication) or no observable trichothecenes.

The stereochemistries at C4′, C6′, and C13′ in a number of these derivatives as well as C3′ in B20, B23, and B24 are not established with certainty. However, baccharinoids B1, B2,[13] B4, B5, B6, and B8[1] and miotoxins A and D[4] all have the same relative configurations at C4′, as illustrated in the structure diagrams for baccharinoids B1 (**17**), B2 (**18**), B4 (**1**), B5 (**3**), B6 (**2**), and B8 (**4**). It seems likely that those other baccharinoids with a C4′ hydroxy group also have this same relative configuration. Attempts to epoxidize baccharinoids B4 and B13 to a common compound (as roridin E was converted to baccharinoid B17) failed. The 4′-OH group apparently deactivates the C2′,C3′ double bond sufficiently so that the dienic system is extensively oxidized by the peracid. In the same fashion, baccharinoids B9 and B10 failed to give baccharinoids B5 and B8, respectively, upon treatment with MCPBA. To date, all of the baccharinoids, miotoxins,[4] and roridins, with the exception of isororidin E,[11] whose absolute configurations have been established with certainty have proven to be *R* at C6′. With the stereochemistry fixed as *R* at C6′ in the baccharinoids, roridins, and miotoxins, the ^{1}H NMR spectra of the acetates can be used to assign the configuration at C13′. Thus, C13′ acetates of those compounds that fall into the erythro series (*S* at C13′) exhibit an eight-line pattern (dd, $J_{13',14'} \approx 6.5$ Hz and $J_{6',13'} \approx 3.5$ Hz) for H13′, whereas acetates of the threo series (*R* at C13′) exhibit a five-line pattern (dd, $J_{6',13'} \approx J_{13',14'} \approx 6.5$ Hz) for H13′ in the ^{1}H NMR spectra. Furthermore, the chemical shifts for H13′ in the acetates are consistently at higher frequencies for the threo series (C13′*R*) than for those observed in the erythro series (C13′*S*). The other consistent pattern observed is in the ^{13}C NMR spectra. The chemical shifts of C13′ in the threo series (e.g., baccharinoids B4, B5, B7, B9, B13, and B23) are at ca. 1–2 ppm higher frequency relative to the C13′ shifts in the erythro series (e.g., baccharinoids B6, B8, B3, B14, and B24). Furthermore, the C6′ carbon resonates at a consistently higher frequency ($\Delta\delta \approx 1.2$ ppm) in the threo series (C13′*R*), and the C14′ methyl resonance also usually resonates at a higher frequency when C13′ is *R*. However, baccharinoids B9/B10 are an exception, since, in this case, C14′ in baccharinoid B10 (C13′*S*) resonates at a frequency higher than that observed for C14′ in baccharinoid B9 (C13′*R*).

Conclusions

The origin of these trichothecene mycotoxins in *B. megapotamica* and *B. coridifolia* is uncertain, but there is strong evidence, albeit circumstantial, that the plants acquire these toxins from a fungal source.[4,6] The isolation of trichoverrols A and B (**19**), which are believed to be biosynthetic intermediates leading to macrocyclic trichothecenes in *Myrothecium* species,[15] lends further support to this idea. Furthermore, in other studies,[16] we have shown that several other *Baccharis* species growing in the stand of *B. megapotamica* contain no macrocyclic trichothecenes. In addition, the presence of roridins A, D, and E in *B. coridifolia* depends upon where the plant is collected.[16] Thus, collections of this plant separated by only a few kilometers differ markedly in that some have very high concentrations of roridins (>100 ppm) whereas others have none. Other *B. coridifolia* plants collected several hundred kilometers away also were shown to contain high concentrations of roridins.[16] It should be noted that these data are for fresh plant material.[16] The isolation work reported in this paper was on plant material that had been in storage for over two years. Examination of this dried, stored plant material made it clear that there was no observable microbial activity that could account for the presence of the toxins. These data are consistent with the idea that in order for the *Baccharis* plants to have these toxins, there must be a trichothecene-producing fungus near the roots of the plants. A survey of the soils associated with these *Baccharis* plants has turned up isolates of *Myrothecium* species.[4,16] However, in our hands, most of these isolates under laboratory conditions did not produce macrocyclic trichothecenes, and the few that did produce these toxins did so only in poor yield.[16] Thus, the details of origin of the macrocyclic trichothecenes in *B. megapotamica* and *B. coridifolia* remain obscure.

Experimental Section

General. Melting points were determined on a Fisher-Johns hot-stage melting point apparatus and are uncorrected. Infrared spectra were determined in chloroform or dichloromethane on a Perkin-Elmer Model 281 spectrometer. Ultraviolet spectra were determined in methanol on a Perkin-Elmer Model 552 spectrophotometer. Optical rotations were determined on a Perkin-Elmer 241 automatic polarimeter. Nuclear magnetic resonance spectra were determined in $CDCl_3$ on an IBM SY-200 MHz spectrometer with tetramethylsilane 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 ammonia as a reagent gas.

Thin-layer chromatography (TLC) was performed on precoated TLC plates of silica gel 60F-254 (0.2 mm). Visualization was done by viewing the developed plates under short-wavelength UV light and by spraying with vanillin/sulfuric acid.[17] The Model 7942 Chromatotron (Harrison Research Laboratories) was used for preparative TLC with the plates, prepared according to instructions in the manual, of 1-, 2-, or 4-mm thickness, using E. Merck silica gel or alumina on regular glass circular disks.

Filtration chromatography was done with flash grade silica gel (230–400 mesh, E. Merck) or alumina (activity III, neutral, Woelm) in a sintered glass funnel. Medium-pressure and flash chromatographies were done on columns packed with Whatman LPS-1 or Adsorbosil silica and flash grade silica (230–400 mesh), respectively, under standard conditions. Separations with medium-pressure chromatography were monitored with an ISCO UV5-A detector. HPLC was done on an Altex Model 332 gradient HPLC. For normal-phase HPLC, a Spherisorb silica (5 μm, 25 cm × 4.6 mm) column and a Spherisorb amino (5 μm, 25 cm × 4.6 mm) column were used for analytical injections. For preparative HPLC, Supelco Inc. (5 μm, 25 cm × 10 mm) semipreparative and Zorbax (5 μm, 25 cm × 20 mm) preparative columns were employed.

Large-Scale Extract/Workup of Plant Material. (a) 2-Propanol Extraction. The primary extraction of the 1800 kg of dried plant material (upper portion of plant) was extracted with 2-propanol at the Frederick Cancer Research Center in Frederick, MD. The ground plant material was extracted in four different portions. Each portion was subjected to three hot (50–55 °C) percolations with 500 gal of 2-propanol. Each percolation lasted for 24 h, and a total of 6000 gal of 2-propanol was used for the extraction. The 2-propanol extracts were concentrated to four drums of concentrate (ca. 200 gal), which were concentrated further under high vacuum to give ca. 100 kg of dark black tar.

(b) Hexane/Methanol–Water (90/10) Partitioning. The hexane/MeOH–water partitioning of the 100 kg of native extract

(16) Jarvis, B. B.; Wells, K. M.; Lee, Y.-W.; Bean, G. A.; Kommedahl, T.; Lombardo de Barros, C. S.; Barros, S. S. *Phytopathology*, in press.

(17) Jarvis, B. B.; Pavanasasivam, G.; Bean, G. A. In *Trichothecenes and Other Mycotoxins*; Lacey, J., Ed.; Academic: London, 1985; p 221.

was carried out in three portions with use of a total of 361 gal of methanol, 37.8 gal of water, and 330 gal of hexane. Each portion of native extract was taken up in the aqueous methanol and washed twice with hexane.

(c) Ferric Chloride Gel Treatment. The MeOH/water solubles in 405 gal of solution were treated with ferric chloride gel in three portions as follows: A 750-gal stainless steel tank having a PVC liner was partially filled with 372 gal of deionized water. To this was added 155 lb of 30% ferric chloride solution (Coyne Chemical Co.), with good stirring. The pH of this solution was adjusted to 4.0–4.5 by the addition of 50% NaOH (ca. 5.5 gal). When the pH was stable, 135 gal of the methanol/water solubles from the hexane partitions was added, and the mixture was stirred vigorously for 2 h, and toward the end of this stirring period, 32 gal of methanol was added. The brown syrup-like suspension was filtered in large porcelain "Nutches" (Büchner funnels). As a filter became filled with brown residue (chocolate-pudding-like), the residue was washed well with 50% aqueous methanol and then discarded. The clear light yellow filtrate, which contained the baccharinoids, was concentrated on a 20-L rotary evaporator at 40 °C under vacuum, to remove most of the methanol to give ca. 920 gal (total) of concentrated light yellow solution.

(d) Dichloromethane/Water Partitioning. The partitioning of the ferric chloride gel filtrate with dichloromethane was carried out in six portions. The following is a description of one of these six portions: A 225-gal stainless steel conical-bottom jacketed tank, equipped with a glass plug valve in the bottom drain, was used as a separatory funnel. The concentrate (155 gal) from the ferric chloride gel treatment was pumped into this vessel along with 35 gal of dichloromethane. This mixture was stirred vigorously for 2 h and then allowed to separate overnight. The lower dichloromethane layer was drained (27 gal), and the upper layer was extracted three more times with 35 gal each of dichloromethane. All of the extracts were combined and concentrated to give 4.7 kg (total) of dark brown oil.

(e) Large-Scale Silica Gel Chromatography. The silica gel chromatography of the concentrate from (c) was carried out in two 6 in. × 10 ft stainless steel columns in series packed with 136 lb of Davisil 633 silica gel (200–400 mesh) in CH_2Cl_2 and then conditioned by passing 50 gal of 50/50 hexane/dichloromethane through the columns. The sample (4.7 kg) was dissolved in 2.5 gal of CH_2Cl_2 and pumped onto the top of the first column. The following solvent gradient (flow ca. 15 gal/h) was then passed through the column: 50/50 CH_2Cl_2/hexane (98 gal); 100% CH_2Cl_2 (100 gal); 1% methanol in CH_2Cl_2 (100 gal); 3% methanol in CH_2Cl_2 (100 gal); 5% methanol in CH_2Cl_2 (38 gal); 7% methanol in CH_2Cl_2 (55 gal); 10% methanol in CH_2Cl_2 (40 gal); 100% methanol (40 gal). The eluent was collected in 5-gal fractions, which were combined on the basis of their TLC properties to give fractions I–X shown in Scheme I.

Fraction VII (1125 g) was chromatographed through a 6 in. × 10 ft stainless steel column packed with 63.8 lb of Davisil 633 silica gel (200–400 mesh) in CH_2Cl_2 with the following solvent gradient (flow rate ca. 19 gal/h and an inlet pressure of 80–85 psi): 1% methanol in CH_2Cl_2 (38 gal); 2% methanol in CH_2Cl_2 (20 gal); 2.6% methanol in CH_2Cl_2 (30 gal); 3% methanol in CH_2Cl_2 (129 gal); 10% methanol in CH_2Cl_2 (25 gal). Fractions (5 gal) were collected and examined by TLC. When the desired B5/B8 mixture started coming off the column, smaller fractions (2.5 gal) were collected. Like fractions were combined and concentrated to give fractions VII-A to -E (Scheme I).

NaOH Treatment. The crude fractions were dissolved in dichloromethane, 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 phase was decanted, and the process was repeated, usually a total of three times, until the aqueous phase was light yellow in color. If the phases were mixed in the normal fashion in a separatory funnel, intractable emulsions resulted. After separation of the organic phase, the aqueous layer was washed carefully with dichloromethane twice. The dichloromethane layers were combined, dried ($MgSO_4$), and concentrated in vacuo.

Acetylation of Trichothecenes. The trichothecene dissolved in dichloromethane (usually 5–10 mg in 1 mL of CH_2Cl_2) was mixed with an excess amount of acetic anhydride (ca. 5 equiv) and triethylamine (ca. 10 equiv) with a catalytic amount of (*N*,*N*-dimethylamino)pyridine (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).

Table II. R_f Values for Baccharinoids on Silica Gel TLC

baccharinoid	R_f values in solvents A[a]	B[b]	C[c]
B9	0.40	0.43	0.48
B10	0.40	0.36	0.43
B12	0.54	0.76	0.65
B13	0.19	0.28	0.43
B14	0.19	0.21	0.42
B16	0.10	0.17	0.29
B17	0.66	0.74	0.54
B20	0.43	0.38	0.46
B21	0.50	0.57	0.48
B23	0.17	0.20	0.31
B24	0.16	0.18	0.28
B25	0.31	0.64	0.58
B27	0.44	0.44	0.51

[a] 4% MeOH/CH_2Cl_2. [b] EtOAc. [c] 30% *i*-PrOH/hexane.

TLC Data. Table II gives the TLC data for the new trichothecenes isolated.

Isolation of Baccharinoid B17 (7). After NaOH treatment of fraction VI, the CH_2Cl_2 solubles (160 g) were divided into four parts. Each part (ca. 40 g) in 100 mL of CH_2Cl_2 was subjected to a filtration chromatography on alumina (ca. 140 g) and eluted with (i) 10% 2-propanol/hexane, 3 L, (ii) 100% 2-propanol, 2 L, and (iii) 10% MeOH/2-propanol, 1 L, to give three fractions, which were 20 g, 4.0 g, and 1.0 g, respectively. Fraction ii (16.5 g total) was passed through a column containing Sephadex LH-20 with CH_2Cl_2 as eluent to give a trichothecene-containing fraction (13.3 g), which was loaded on a flash chromatography column packed with silica gel (280 g) and eluted with 30–100% ethyl acetate in hexane. The trichothecene-containing fraction (1.7 g) from the flash chromatography was put on a 4-mm Chromatotron plate (SiO_2; 0–1.5% MeOH/CH_2Cl_2 as eluent) to give three fractions: (a) 200 mg, (b) 850 mg, and (c) 600 mg. Crystallization of fraction b from CH_2Cl_2/ether gave roridin D (**6**). Recrystallization from CH_2Cl_2/ether yielded 550 mg of **6**, whose properties matched those reported earlier.[18] Fraction c was further purified by PTLC on the Chromatotron (2-mm SiO_2 plate; 0–1.5% MeOH/CH_2Cl_2 as eluent). This separation gave roridin D (**6**, 10 mg) together with a fraction containing a new trichothecene, which was crystallized from CH_2Cl_2/ether to give baccharinoid B17 (**7**, 75 mg): mp >300 °C; $[\alpha]^{25}_D$ +11.0 ± 0.7 (*c* 0.68, CH_2Cl_2); UV λ_{max} 260 nm; IR ($CHCl_3$) 3580, 2880, 1755, 1720, 1170, 1100 cm^{-1}; ^1H NMR δ 0.77 (3 H, s, 14-H), 1.23 (3 H, d, J = 6.5 Hz, 14′-H), 1.37 (3 H, s, 12′-H), 1.66 (3 H, s, 16-H), 2.19 (1 H, ddd, $J_{2,3\beta} = J_{3\beta,4}$ = 4.6 Hz and J_{gem} = 15.3 Hz, 3β-H), 2.4–2.6 (3 H, m, 3α-H and 4′-H), 2.76, 3.17 (1 H each, AB pattern, J = 3.9 Hz, 13-H), 3.09 (1 H, d, J = 5.4 Hz, 10-H), 3.30 (1 H, s, 2′-H), 3.32–3.38 (1 H, m, 5′B-H), 3.57 (1 H, d, J = 5.4 Hz, 11-H), 3.84–3.92 (1 H, m, 5′A-H), 3.94 (1 H, d, J = 5.0 Hz, 2-H), 4.20, 4.43 (1 H each, AB pattern, J = 12.6 Hz, 15-H), 5.76 (1 H, dd, J = 4.6 and 8.2 Hz, 4-H), 5.80 (1 H, d, J = 11.2 Hz, 10′-H), 5.97 (1 H, dd, J = 3.0 and 15.6 Hz, 7′-H), 6.60 (1 H, dd, $J_{8',9'} = J_{9',10'}$ = 11.2 Hz, 9′-H), 7.54 (1 H, dd, J = 11.2 and 15.6 Hz, 8′-H); mass spectrum [negative-ion chemical ionization (NICI), ammonia reagent gas] calcd for $C_{29}H_{38}O_{10}$ m/e 546.2465, found 546.2443.

B17 acetate: ^1H NMR δ 0.75 (3 H, s, 14-H), 1.17 (3 H, d, J = 6.4 Hz, 14′-H), 1.34 (3 H, s, 12′-H), 1.62 (3 H, s, 16-H), 2.02 (3 H, s, CH_3COO), 2.18 (1 H, ddd, $J_{2,3\beta} = J_{3\beta,4}$ = 4.8 Hz and J_{gem} = 15.2 Hz, 3β-H), 2.4–2.6 (3 H, m, 3α-H and 4′-H), 2.74, 3.14 (1 H each, AB pattern, J = 4.0 Hz, 13-H), 3.07 (1 H, d, J = 5.2 Hz, 10-H), 3.26 (1 H, s, 2′-H), 3.54 (1 H, d, J = 5.2 Hz, 11-H), 3.91 (1 H, d, J = 4.8 Hz, 2-H), 4.17, 4.43 (1 H each, AB pattern, J = 12.4 Hz, 15-H), 5.03 (1 H, dq, $J_{6',13'} = J_{13',14'}$ = 6.4 Hz, 13′-H), 5.72 (1 H, dd, J = 4.8 and 8.0 Hz, 4-H), 5.77 (1 H, d, J = 11.5 Hz, 10′-H), 5.89 (1 H, dd, J = 3.1 and 15.4 Hz, 7′-H), 6.58 (1 H, dd, $J_{8',9'}$ =

(18) Bonner, B.; Tamm, C. *Helv. Chim. Acta* **1966**, *49*, 2547.

$J_{9',10'}$ = 11.5 Hz, 9'-H), 7.45 (1 H, dd, J = 11.5 and 15.4 Hz, 8'-H); mass spectrum (NICI, ammonia reagent gas) calcd for $C_{31}H_{40}O_{11}$ m/e 588.2571, found 588.2553.

Epoxidation of Roridin D (6) with *m*-Chloroperoxybenzoic Acid (MCPBA). Roridin D (6) was epoxidized to yield 9β,10β-epoxyroridin D in 85% yield, following the procedure of Jarvis et al.[19] 9β,10β-Epoxyroridin D has spectral and physical properties identical to those of baccharinoid B17 (9).

Isolation of Baccharinoid B12 (8). The dichloromethane solubles (172.0 g) obtained from the NaOH treatment of fraction VII-B (Scheme I) were divided into four parts. Each part (ca. 45 g) was placed on top of a bed of alumina (ca. 140 g) in a sintered glass funnel, and solvent was pulled through the system under aspirator pressure. Three fractions were collected: (i) 3 L of 15% 2-propanol/hexane, 20 g, (ii) 2 L of 100% 2-propanol, 1.3 g, and (iii) 500 mL of 100% methanol, 5.0 g. Fraction ii (5.4 g total) was triturated with ether/CH_2Cl_2 to give a precipitate. Recrystallization from acetone/hexane gave pure baccharinoid B12 (8, 430 mg): mp 248–250 °C; $[\alpha]^{25}_D$ +12.6 ∓ 1.0 (c 0.54, MeOH); UV λ_{max} 260 nm; IR ($CHCl_3$) 3500, 2990, 1730, 1715, 1180, 1100 cm^{-1}; ^{1}H NMR δ 0.85 (3 H, s, 14-H), 1.19 (3 H, d, J = 6.4 Hz, 14'-H), 1.46 (3 H, s, 12'-H), 1.75 (3 H, s, 16-H), 2.79, 3.08 (1 H each, AB pattern, J = 4.0 Hz, 13-H), 3.30 (1 H, s, 2'-H), 3.40–3.50 (1 H, m, 5'B-H), 3.62–3.75 (5 H, m, 2-H, 11-H, 5'A-H, 6'-H, and 13'-H), 4.18 (1 H, d, J = 5.6 Hz, 11-H), 4.19, 4.43 (1 H each, AB pattern, J = 12.6 Hz, 15-H), 4.35 (1 H, dd, J = 2.7 and 5.0 Hz, 3β-H), 5.57 (1 H, d, J = 5.6 Hz, 10-H), 6.02 (1 H, dd, J = 2.7 and 15.6 Hz, 7'-H), 6.63 (1 H, dd, $J_{8',9'}$ = $J_{9',10'}$ = 11.5 Hz, 9'-H), 7.50 (1 H, dd, J = 11.5 and 15.6 Hz, 8'-H); mass spectrum (NICI, ammonia reagent gas) calcd for $C_{29}H_{38}O_{10}$ m/e 546.2465, found 546.2442.

B12 diacetate: ^{1}H NMR δ 0.83 (3 H, s, 14-H), 1.20 (3 H, d, J = 6.5 Hz, 14'-H), 1.62 (3 H, s, 12'-H), 1.74 (3 H, s, 16-H), 2.08, 2.14 (3 H each, s, CH_3COO), 2.80, 3.06 (1 H each, AB pattern, J = 4.0 Hz, 13-H), 3.29 (1 H, s, 2'-H), 3.30–3.40 (1 H, m, 5'B-H), 3.86 (1 H, d, J = 4.4 Hz, 11-H), 3.70–3.85 (1 H, m, 6'-H), 3.90 (1 H, d, J = 4.9 Hz, 2-H), 3.99 (1 H, br s, 5'A-H), 4.36, 4.46 (1 H each, AB pattern, J = 12.5 Hz, 15-H), 5.06 (1 H, dq, $J_{6',13'}$ = $J_{13',14'}$ = 6.5 Hz, 13'-H), 5.33 (1 H, dd, $J_{3,4}$ = 3.7 Hz and $J_{2,3}$ = 4.9 Hz, 3β-H), 5.47 (1 H, d, J = 4.4 Hz, 10-H), 5.64 (1 H, d, J = 3.7 Hz, 4-H), 5.84 (1 H, d, J = 11.3 Hz, 10'-H), 5.93 (1 H, dd, $J_{6',7'}$ = 3.3 Hz and $J_{7',8'}$ = 15.6 Hz, 7'-H), 6.64 (1 H, dd, $J_{8',9'}$ = $J_{9',10'}$ = 11.3 Hz, 9'-H), 7.43 (1 H, dd, J = 11.3 and 15.6 Hz, 8'-H); mass spectrum (NICI, ammonia reagent gas) calcd for $C_{33}H_{42}O_{12}$ m/e 630.2676, found 630.2630.

Hydrolysis of Baccharinoid B12 (8). Into a solution of 8 (20.2 mg) in 1.5 mL of absolute methanol was added anhydrous sodium methoxide (21.0 mg; 10 equiv) in 2 mL of methanol. The reaction mixture was stirred at room temperature for 8 h and passed through an acidic resin (Dowex 50 W). The solvent was evaporated in vacuo. The product was purified on the Chromatotron (1-mm SiO_2 plate; 5–7% MeOH/CH_2Cl_2 as eluent). Without further purification, the hydrolysis product of 8 was acetylated as described above. Crystallization from hexane gave triacetylated product whose properties were identical to those of **10**, the triacetate of scirpentriol.[12]

Isolation of Baccharinoid B21 (11). Fraction VII-C (Scheme I) (294 g) was subjected to preparative HPLC (SiO_2, 3.5% MeOH in CH_2Cl_2; Waters Prep. LC/500 A; 2 in. × 2 ft column) to give 10 fractions. The third fraction (4.0 g) was subjected to MPLC (155 g of silica, 50–100% ethyl acetate/hexane eluent) to give two fractions, A (0.3 g) and B (1.5 g). Fraction B was further fractionated on the Chromatotron (4-mm SiO_2 plate) with 50% ethyl acetate/hexane to give a compound whose properties are identical to those of baccharinoid B5 (3, 560 mg) in all respects. Fraction A was put on a 2-mm SiO_2 plate on the Chromatotron and eluted with 50–100% ethyl acetate/hexane to give a fraction, which was crystallized from CH_2Cl_2/ether to give baccharinoid B21 (11), 140 mg); mp 259–260 °C; $[\alpha]^{25}_D$ +73.5 ∓ 1.0 (c 0.68, CH_2Cl_2); UV λ_{max} 260 nm; IR 2880, 1750, 1715, 1170, 1100 cm^{-1}; ^{1}H NMR δ 0.82 (3 H, s, 14-H), 1.18 (3 H, d, J = 6.0 Hz, 14'-H), 1.55 (3 H, s, 12'-H), 1.81 (3 H, s, 16-H), 2.10–2.22 (3 H, m, 3β-H and 7-H), 2.44 (1 H, dd, J_{gem} = 15.4 Hz and $J_{3\alpha,4}$ = 8.3 Hz, 3α-H), 2.82, 3.11 (1 H each, AB pattern, J_{AB} = 4.0 Hz, 13-H), 3.29 (1 H, s, 2'-H), 3.33–3.37 (1 H, m, 5'B-H), 3.58–3.64 (2 H, m, 11-H and 5'A-H), 3.64–3.80 (2 H, m, 6'-H and 13'-H), 3.85 (1 H, d, J = 4.9 Hz, 2-H), 3.82–3.99 (1 H, m, 8-H), 4.19, 4.40 (1 H each, AB pattern, J_{AB} = 12.3 Hz, 15-H), 5.48 (1 H, d, J = 5.3 Hz, 10-H), 5.75 (1 H, dd, J = 4.3 Hz and J = 8.3 Hz, 4-H), 5.78 (1 H, d, J = 11.4 Hz, 10'-H), 5.95 (1 H, dd, $J_{6',7'}$ = 2.9 Hz and $J_{7',8'}$ = 15.7 Hz, 7'-H), 6.58 (1 H, dd, $J_{9',10'}$ = $J_{8',9'}$ = 11.4 Hz), 7.48 (1 H, dd, $J_{7',8'}$ = 15.7 Hz and $J_{8',9'}$ = 11.4 Hz, 8'-H); mass spectrum (NICI, ammonia reagent gas) calcd for $C_{29}H_{38}O_{10}$ m/e 546.2465, found 546.2466.

B21 diacetate: ^{1}H NMR δ 0.80 (3 H, s, 14-H), 1.17 (3 H, d, J = 6.5 Hz, 14'-H), 1.58 (3 H, s, 12'-H), 1.70 (3 H, s, 16-H), 2.02, 2.06 (3 H each, s, CH_3COO), 2.15–2.39 (3 H, m, 3β-H and 7-H), 2.45 (1 H, dd, J = 8.3 and 15.6 Hz, 3α-H), 2.78, 3.10 (1 H each, AB pattern, J = 4.0 Hz, 13-H), 3.37 (1 H, s, 2'-H), 3.44–3.54 (1 H, m, 5'B-H), 3.62 (1 H, d, J = 5.3 Hz, 11-H), 3.94 (1 H, br s, 5'A-H), 4.20, 4.47 (1 H each, AB pattern, J = 12.4 Hz, 15-H), 5.02 (1 H, dq, $J_{6',13'}$ = $J_{13',14'}$ = 6.5 Hz, 13'-H), 5.15 (1 H, dd, J = 4.9 and 9.8 Hz, 8-H), 5.55 (1 H, d, J = 5.3 Hz, 10-H), 5.73 (1 H, dd, J = 4.1 and 8.3 Hz, 4-H), 5.77 (1 H, d, J = 11.4 Hz, 10'-H), 5.88 (1 H, dd, J = 3.2 and 15.6 Hz, 7'-H), 6.58 (1 H, dd, $J_{8',9'}$ = $J_{9',10'}$ = 11.4 Hz, 9'-H), 7.40 (1 H, dd, J = 11.4 and 15.8 Hz, 8'-H); mass spectrum (NICI, ammonia reagent gas) calcd for $C_{33}H_{42}O_{12}$ m/e 630.2676, found 630.2651.

Selenium Dioxide Oxidation of Roridin D (6). Roridin D (6) was oxidized by selenium dioxide according to the method of Jarvis et al.,[19] and the product was isolated by PTLC on the Chromatotron (1-mm SiO_2 plate, 2% MeOH in CH_2Cl_2 as eluent) to give a 35% yield of baccharinoid B21 (11).

Fractionation of VII-E1a. Fraction VII-E1a (20.2 g, Scheme II) was loaded on an MPLC column packed with silica gel (450 g) and eluted with 3–7% 2-propanol in dichloromethane to give four fractions containing trichothecenes: (i) 2.2 g, (ii) 1.1 g, (iii) 1.6 g, and (iv) 1.7 g (Scheme II). Fraction ii was put on a 4-mm Chromatotron plate (SiO_2) and eluted with 50% ethyl acetate (containing 1% 2-propanol) in hexane to give a fraction A (50 mg) containing a trichothecene whose properties are identical to those of B7 (13) together with a fraction B (810 mg) containing five different trichothecenes. Fraction B was further fractionated by PTLC on the Chromatotron by using a 2-mm alumina plate with 3–8% 2-propanol/dichloromethane as eluent. Five fractions were obtained: baccharinoids B1 (20 mg), B2 (25 mg), B3 (38 mg), B4 (52 mg), and B5 (130 mg). Fraction iii was subjected to PTLC on the Chromatotron (4-mm SiO_2 plate, 1–4% 2-propanol/CH_2Cl_2) to give two fractions C (70 mg) and D (92 mg). These fractions were later combined with others (vide infra) for further purification.

Fraction VII-E2b (5.4 g, Scheme II) was subjected to filtration chromatography (110 g of SiO_2, 15–100% ethyl acetate in hexane as eluent) to give two fractions E (1.1 g) and F (3.9 g). An MPLC separation of fraction F gave three trichothecene-containing fractions: F1 (90 mg), F2 (175 mg), and F3 (155 mg). PTLC of F2 on the Chromtotron (2-mm alumina plate, 3–8% 2-propanol/CH_2Cl_2) gave F2a (82 mg) and F2b (78 mg). In a like manner, PTLC of F3 gave F3a (45 mg) and F3b (54 mg).

On the basis of TLC analysis, fractions B3 and F3a were combined and crystallized from chloroform/ether. Recrystallization from CH_2Cl_2/ether gave 65 mg of baccharinoid B10 (15): mp 157–158 °C; $[\alpha]^{25}_D$ +8.1 (c 0.62, $CHCl_3$); UV λ_{max} 220, 260 nm; IR ($CHCl_3$) 3420, 1720, 1650, 1610, 1170 cm^{-1}; ^{1}H NMR δ 0.72 (3 H, s, 14-H), 1.19 (3 H, d, J = 6.4 Hz, 14'-H), 1.33 (3 H, s, 16-H), 2.33 (3 H, d, J = 1.0 Hz, 12'-H), 2.43 (1 H, dd, J = 8.0 and 5.2 Hz, 3α-H), 2.74, 3.18 (1 H each, AB pattern, J = 4.0 Hz, 13-H), 3.08 (1 H, d, J = 5.2 Hz, 10-H), 3.60 (1 H, d, J = 5.2 Hz, 11-H), 3.90 (1 H, d, J = 4.9 Hz, 2-H), 3.81, 4.45 (1 H each, AB pattern, J = 12.7 Hz, 15-H), 5.71 (1 H, d, J = 11.4 Hz, 10'-H), 5.87 (1 H, dd, J = 3.0 and 15.6 Hz, 7'-H), 5.97 (1 H, br s, 2'-H), 5.98 (1 H, dd, J = 4.2 and 8.0 Hz, 4-H), 6.53 (1 H, dd, $J_{8',9'}$ = $J_{9',10'}$ = 11.4 Hz, 9'-H), 7.34 (1 H, dd, J = 11.4 and 15.6 Hz, 8'-H); mass spectrum (positive-ion CI, methane reagent gas) calcd for $C_{29}H_{38}O_{10}$ + H m/e 547.2544, found 547.2532.

B10 diacetate: ^{1}H NMR δ 0.74 (3 H, s, 14-H), 1.20 (3 H, d, J = 6.5 Hz, 14'-H), 1.35 (3 H, s, 16-H), 2.08, 2.12 (3 H each, s, CH_3COO), 2.31 (3 H, d, J = 1.2 Hz, 12'-H), 2.78, 3.16 (1 H each, AB pattern, J = 4.0 Hz, 13-H), 3.92 (1 H, d, J = 5.0 Hz, 2-H), 3.75, 4.68 (1 H each, AB pattern, J = 12.4 Hz, 15-H), 5.00 (1 H, dq, $J_{13',14'}$ = 6.5 Hz, $J_{6',13'}$ = 3.5 Hz, 13'-H), 5.23 (1 H, m, 4'-H),

(19) Jarvis, B. B.; Midiwo, J. O.; Mazzola, E. P. *J. Med. Chem.* **1984**, *27*, 239.

5.73 (1 H, d, J = 11.3 Hz, 10′-H), 6.53 (1 H, dd, $J_{8',9'} = J_{9',10'}$ = 11.3 Hz, 9′-H), 7.37 (1 H, dd, J = 11.3 and 15.0 Hz, 8′-H).

Isolation of Baccharinoid B9 (14). On the basis of TLC analysis, fractions B4, C (from fractionation of VII-E1a), and F3b (from fractionation of VII-E2b) were combined and crystallized from dichloromethane/ether to give 145 mg of pure baccharinoid B9 (**14**): mp 216–218 °C; $[\alpha]^{25}_D$ +2.4 ∓ 0.8 (c 0.68, CH_2Cl_2); UV λ_{max} 220, 262 nm; IR ($CHCl_3$) 2930, 2870, 1720, 1150, 1095 cm^{-1}; ^1H NMR δ 0.69 (3 H, s, 14-H), 1.18 (3 H, d, J = 6.0 Hz, 14′-H), 1.33 (3 H, s, 16-H), 2.10–2.25 (1 H, m, 3β-H), 2.31 (3 H, d, J = 1.2 Hz, 12′-H), 2.45 (1 H, dd, J = 8.5 and 15.8 Hz, 3α-H), 2.74, 3.14 (1 H each, AB pattern, J = 4.0 Hz, 13-H), 3.07 (1 H, d, J = 5.2 Hz, 10-H), 3.63–3.73 (6 H, m, 4′-H, 5′-H, 6′-H, 13′-H, and 11-H), 3.90 (1 H, d, J = 5.0 Hz, 2-H), 3.76, 4.46 (1 H each, AB pattern, J = 12.7 Hz, 15-H), 5.71 (1 H, d, J = 11.3 Hz, 10′-H), 5.87 (1 H, dd, J = 2.5 and 16.1 Hz, 7′-H), 5.98 (2 H, m, 2′-H and 4-H), 6.53 (1 H, dd, $J_{8',9'} = J_{9',10'}$ = 11.3 Hz, 9′-H), 7.38 (1 H, dd, J = 11.3 and 16.1 Hz, 8′-H); mass spectrum (NICI, ammonia reagent gas) calcd for $C_{29}H_{38}O_{10}$ m/e 546.2465, found 546.2443.

B9 diacetate: ^1H NMR δ 0.74 (3 H, s, 14-H), 1.17 (3 H, d, J = 6.5 Hz, 14′-H), 1.35 (3 H, s, 16-H), 2.06, 2.13 (3 H each, s, CH_3COO), 2.31 (3 H, d, J = 1.2 Hz, 12′-H), 2.41 (1 H, dd, J = 8.3 and 15.6 Hz, 3α-H), 2.77, 3.15 (1 H each, AB pattern, J = 4.0 Hz, 13-H), 3.07 (1 H, d, J = 5.1 Hz, 10-H), 3.46–3.51 (1 H, m, 5′B-H), 3.69–3.76 (1 H, m, 6′-H), 3.91 (1 H, d, J = 4.8 Hz, 2-H), 3.95–4.03 (1 H, m, 5′A-H), 3.72, 4.70 (1 H each, AB pattern, J = 12.6 Hz, 15-H), 5.13 (1 H, dq, $J_{6',13'} = J_{13',14'}$ = 6.5 Hz, 13′-H), 5.22 (1 H, t, J = 4.0 Hz, 4′-H), 5.73 (1 H, d, J = 11.3 Hz, 10′-H), 5.75–5.84 (2 H, m, 2′-H and 4-H), 5.89 (1 H, dd, J = 3.3 and 15.6 Hz, 7′-H), 6.56 (1 H, dd, $J_{8',9'} = J_{9',10'}$ = 11.3 Hz, 9′-H), 7.38 (1 H, dd, J = 11.3 and 15.6 Hz, 8′-H); mass spectrum (NICI, ammonia gas reagent) calcd for $C_{33}H_{42}O_{12}$ m/e 630.2676, found 630.2693.

Isolation of Baccharinoid B20 (16). On the basis of TLC analysis, fractions B5, D (from fractionation of VII-E1a), F1, and F2a (from fractionation of VII-E2b) were combined and crystallized from CH_2Cl_2/ether. Recrystallization from acetone/hexane gave 210 mg of pure baccharinoid B20 (**16**): mp 170–172 °C; $[\alpha]^{25}_D$ +39.9 ∓ 0.4 (c 1.44, $CHCl_3$); UV λ_{max} 262 nm; IR (CH_2Cl_2) 2940, 1730, 1370 cm^{-1}; ^1H NMR δ 0.69 (3 H, s, 14-H), 0.98 (3 H, d, J = 6.6 Hz, 12′-H), 1.16 (3 H, d, J = 6.5 Hz, 14′-H), 1.34 (3 H, s, 16-H), 2.05–2.21 (1 H, m, 3β-H), 2.35–2.50 (2 H, m, 3α-H and 3′-H), 2.74, 3.14 (1 H each, AB pattern, J = 4.0 Hz, 13-H), 3.06 (1 H, d, J = 5.1 Hz, 10-H), 3.54 (1 H, d, J = 5.1 Hz, 11-H), 3.60–3.68 (3 H, m, 4′-H and 5′-H), 3.80–4.00 (3 H, m, 2-H, 6′-H, and 13′-H), 3.78, 4.64 (1 H each, AB pattern, J = 12.3 Hz, 15-H), 5.74 (1 H, d, J = 11.1 Hz, 10′-H), 5.74 (1 H, m, 4-H), 6.05 (1 H, dd, J = 3.4 and 16.4 Hz, 7′-H), 6.71 (1 H, dd, $J_{8',9'} \approx J_{9',10'}$ = 11.1 Hz, 9′-H), 7.77 (1 H, dd, J = 11.1 and 16.4 Hz, 8′-H); mass spectrum (NICI, ammonia reagent gas) calcd for $C_{29}H_{40}O_{10}$ m/e 548.2621, found 548.2593.

B20 diacetate: ^1H NMR δ 0.73 (3 H, s, 14-H), 1.10 (3 H, d, J = 6.8 Hz, 12′-H), 1.21 (3 H, d, J = 6.5 Hz, 14′-H), 1.36 (3 H, s, 16-H), 2.06, 2.11 (3 H each, s, CH_3COO), 2.05–2.20 (1 H, m, 3β-H), 2.30–2.70 (2 H, m, 3α-H and 3′-H), 2.76, 3.15 (1 H each, AB pattern, J = 4.0 Hz, 13-H), 3.08 (1 H, d, J = 5.4 Hz, 10-H), 3.56 (1 H, d, J = 5.4 Hz, 11-H), 3.56, 3.70 (1 H each, d of AB pattern, J = 4.8 and 10.2 Hz, 5′-H), 3.90–3.96 (2 H, m, 2-H and 6′-H), 3.91, 4.61 (1 H each, AB pattern, J = 12.3 Hz, 15-H), 4.83 (1 H, dd, J = 5.1 and 9.8 Hz, 4′-H), 4.92 (1 H, dq, $J_{6',13'}$ = 4.0 Hz and $J_{13',14'}$ = 6.5 Hz, 13′-H), 5.78 (1 H, d, J = 11.3 Hz, 10′-H), 5.78 (1 H, dd, J = 3.4 and 7.0 Hz, 4-H), 5.93 (1 H, dd, J = 3.3 and 15.5 Hz, 7′-H), 6.60 (1 H, dd, $J_{8',9'} = J_{9',10'}$ = 11.3 Hz, 9′-H), 7.59 (1 H, dd, J = 11.3 and 15.5 Hz, 8′-H); mass spectrum (NICI, ammonia reagent gas) calcd for $C_{33}H_{44}O_{12}$ m/e 632.2833, found 632.2812.

Isolation of Baccharinoids B13 (20), B14 (21), B23 (22), B24 (23), B25 (24), and B27 (25). A large-scale silica gel column run was made with fraction IX (Scheme I) on a 6 in. × 10 ft stainless steel column packed with 63.8 lb of Davisil 633 silica gel and conditioned by pumping 40 gal of 3% methanol in CH_2Cl_2 through the column. The sample (1157 g) was dissolved in 0.5 gal of 4% MeOH in CH_2Cl_2 and pumped onto the column with the following solvent gradient (flow ca. 15–18 gal/h): 3% methanol in CH_2Cl_2 (65 gal); 4% methanol in CH_2Cl_2 (43 gal); 4.5% methanol in CH_2Cl_2 (41 gal); 5% methanol in CH_2Cl_2 (108 gal); 13% methanol in CH_2Cl_2 (40 gal). Like fractions were combined and concentrated under reduced pressure at 50 °C to give fractions IX-A–IX-E (Scheme III). Fraction IX-D was dissolved in CH_2Cl_2 (heating), and hexane was added in small portions. After cooling overnight, the mixture was filtered, giving 83.6 g of a tan solid. This material was taken up in 500 mL of CH_2Cl_2 and carefully washed with 3 × 500 mL of 2.5% aqueous NaOH. The CH_2Cl_2 fraction was dried (Na_2SO_4) and concentrated to give 42 g of gum, which was subjected to preparative HPLC (Waters Prep LC/500) over Woelm alumina (activity III), packed in a 2 ft × 2 in. stainless steel column (flow rate = 150 mL/min, 15% 2-propanol in CH_2Cl_2), to give a series of fractions IX-D1–IX-D6 (Scheme III). Fraction IX-D1 (Scheme III, 12.6 g) was loaded on an MPLC column packed with silica gel (450 g) and eluted with 0–6% methanol in CH_2Cl_2 to give three fractions: A (0.48 g), B (1.06 g), and C (0.30 g). Fraction A was further fractionated by MPLC (60 g of silica gel) with 30–100% ethyl acetate/hexane as eluent. Three fractions were obtained: A1 (34 mg), A2 (28 mg), and A3 (68 mg). Chromatotron separation (1-mm SiO_2 plate, 0–2% MeOH/CH_2Cl_2) of fraction A1 gave a fraction, which was crystallized from CH_2Cl_2/ether. Recrystallization from dichloroethane/hexane gave 11.5 mg of baccharinoid B25 (**24**): mp 205 °C dec; $[\alpha]^{25}_D$ +214 ± 0.4 (c 0.70, MeOH); UV λ_{max} 220, 260 nm; IR (CH_2Cl_2) 1740, 1165 cm^{-1}; ^1H NMR δ 0.81 (3 H, s, 14-H), 1.83 (3 H, s, 16-H), 2.23 (3 H, d, J = 1.2 Hz, 12′-H), 2.10–2.30 (1 H, m, 3β-H), 2.53 (1 H, dd, J = 8.1 and 15.5 Hz, 3α-H), 2.86, 3.17 (1 H each, AB pattern, J = 4.0 Hz, 13-H), 3.88 (1 H, d, J = 5.0 Hz, 2-H), 3.91 (1 H, d, J = 5.4 Hz, 11-H), 4.03–4.14 (3 H, m, 15B-H, 8-H, and 5′B-H), 4.40–4.48 (2 H, m, 4′-H and 5′A-H), 4.17 (1 H, AB pattern, J = 12.7 Hz, 15A-H), 5.56 (1 H, d, J = 5.4 Hz, 10-H), 5.99 (1 H, d, J = 15.8 Hz, 7′-H), 6.07 (1 H, br s, 2′-H), 6.12 (1 H, d, J = 11.3, 10′-H), 6.06–6.11 (1 H, m, 4-H), 6.63 (1 H, dd, $J_{8',9'} = J_{9',10'}$ = 11.3 Hz, 9′-H), 8.11 (1 H, dd, J = 11.3 and 15.8 Hz, 8′-H); mass spectrum (NICI, ammonia reagent gas) calcd for $C_{27}H_{32}O_{10}$ m/e 516.1996, found 516.1989.

B25 diacetate: ^1H NMR δ 0.80 (3 H, s, 14-H), 1.70 (3 H, s, 16-H), 2.10, 2.15 (3 H each, s, CH_3COO), 2.25 (3 H, d, J = 1.3 Hz, 12′-H), 2.00–2.20 (1 H, m, 3β-H), 2.46 (1 H, dd, J = 8.0 and 15.0 Hz, 3α-H), 2.83, 3.17 (1 H each, AB pattern, J = 4.1 Hz, 13-H), 3.83 (1 H, d, J = 5.0 Hz, 11-H), 3.98 (1 H, d, J = 5.0 Hz, 2-H), 4.05–4.16 (1 H, m, 5′B-H), 4.39–4.48 (1 H, m, 5′A-H), 4.03, 4.40 (1 H each, AB pattern, J = 12.8 Hz, 15-H), 5.23–5.38 (2 H, m, 8-H and 4′-H), 5.90–6.04 (2 H, m, 2′-H and 4-H), 6.09 (1 H, d, J = 16.0 Hz, 7′-H), 6.11 (1 H, d, J = 11.0 Hz, 10′-H), 6.63 (1 H, dd, $J_{8',9'} = J_{9',10'}$ = 11.0 Hz, 9′-H), 8.06 (1 H, dd, J = 11.0 and 16.0 Hz, 8′-H); mass spectrum (NICI, ammonia reagent gas) calcd for $C_{31}H_{36}O_{12}$ m/e 600.2208, found 600.2224.

Fraction A3 was crystallized from CH_2Cl_2/ether and recrystallized from dichloroethane/hexane to give 23 mg of baccharinoid B27 (**25**): mp 165 °C dec; $[\alpha]^{25}_D$ +5.4 ∓ 1.0 (c 0.40, MeOH); UV λ_{max} 223, 262 nm; IR (CH_2Cl_2) 3600, 2900, 1720, 1685 cm^{-1}; ^1H NMR δ 0.76 (3 H, s, 14-H), 1.18 (3 H, d, J = 6.4 Hz, 14′-H), 1.83 (3 H, s, 16-H), 2.10 (1 H, ddd, $J_{2,3\beta}$ = 4.9 Hz, $J_{3\beta,4\alpha}$ = 3.9 Hz, and J_{gem} = 15.5 Hz, 3β-H), 2.24 (3 H, d, J = 1.1 Hz, 12′-H), 2.60 (1 H, dd, J = 8.1 and 15.5 Hz, 3α-H), 2.42, 2.94 (1 H each, AB pattern, J = 16.3 Hz, 7-H), 2.85, 3.15 (1 H each, AB pattern, J = 4.0 Hz, 13-H), 3.64, 3.82 (1 H each, d of AB pattern, J = 4.0 and 9.1 Hz, 5′-H), 3.93 (1 H, d, J = 4.9 Hz, 2-H), 4.0–4.31 (6 H, m, 11-H, 15-H, 4′-H, 6′-H, and 13′-H), 5.75 (1 H, d, J = 11.5 Hz, 10′-H), 5.91 (1 H, dd, J = 2.6 and 15.4 Hz, 7′-H), 6.18 (1 H, br s, 2′-H), 6.20 (1 H, dd, J = 3.9 and 8.1 Hz, 4-H), 6.57 (1 H, d, J = 5 Hz, 10-H), 6.58 (1 H, dd, $J_{8'\times,9'} = J_{9',10'}$ = 11.5 Hz, 9′-H), 7.41 (1 H, dd, J = 11.5 and 15.4 Hz, 8′-H); mass spectrum (NICI, ammonia reagent gas) calcd for $C_{29}H_{36}O_{10}$ m/e 544.2308, found 544.2302.

B27 diacetate: ^1H NMR δ 0.80 (3 H, s, 14-H), 1.18 (3 H, d, J = 6.6 Hz, 14′-H), 1.83 (3 H, s, 16-H), 2.07, 2.16 (3 H each, s, CH_3COO), 2.26 (3 H, d, J = 1.2 Hz, 12′-H), 2.56 (1 H, dd, J = 8.0 and 15.6 Hz, 3α-H), 2.64, 2.87 (1 H each, AB pattern, J = 16.4 Hz, 7-H), 2.85, 3.15 (1 H each, AB pattern, J = 3.9 Hz, 13-H) 3.67, 3.91 (1 H each, d of AB pattern, J = 4.6 and 9.8 Hz, 5′-H), 3.93 (1 H, d, J = 5.0 Hz, 2-H), 4.05–4.07 (2 H, m, 11-H and 6′-H), 4.01, 4.43 (1 H each, AB pattern, J = 12.4 Hz, 15-H), 4.99 (1 H, dq, $J_{6',13'}$ = 3.5 Hz and $J_{13',14'}$ = 6.6 Hz, 13′-H), 5.29 (1 H, t, J = 4.6 Hz, 4′-H), 5.75 (1 H, d, J = 11.5 Hz, 10′-H), 5.89 (1 H, dd, J = 3.1 and 16.2 Hz, 7′-H), 5.93 (1 H, br s, 2′-H), 5.97 (1 H, dd, J = 4.3 and 8.1 Hz, 4-H), 6.52 (1 H, d, J = 5.0 Hz, 10-H), 6.56 (1 H, dd, $J_{8',9'} = J_{9',10'}$ = 11.5 Hz, 9′-H), 7.45 (1 H, dd, J = 11.5 and 16.2 Hz, 8′-H); mass spectrum (NICI, ammonia reagent gas) calcd for

$C_{33}H_{40}O_{12}$ *m/e* 628.2520, found 628.2531.

Fraction B was injected onto a preparative HPLC column (silica gel, 16 injections) with ethyl acetate as eluent to give two fractions B1 and B2. Fraction B1 was crystallized from CH_2Cl_2/ether and recrystallized from ethyl acetate/hexane to give 180 mg of baccharinoid B14 (**21**): mp 149–151 °C; $[\alpha]^{25}_D$ +72.4 ± 1.0 (*c* 0.76, MeOH); UV λ_{max} 220, 260 nm; IR (CH_2Cl_2) 3590, 2870, 1715, 1180 cm^{-1}; ^1H NMR δ 0.79 (3 H, s, 14-H), 1.20 (3 H, d, J = 6.4 Hz, 14′-H), 1.83 (3 H, s, 16-H), 2.05–2.20 (1 H, m, 3β-H), 2.27 (3 H, d, J = 1.1 Hz, 12-H), 2.52 (1 H, dd, J = 8.0 and 15.4 Hz, 3α-H), 2.86, 3.16 (1 H each, AB pattern, J = 3.9 Hz, 13-H), 3.66, 3.77 (1 H each, d of AB pattern, J = 3.9 Hz, $J_{4',5B}$ = 4.7 Hz, and J_{gem} = 9.8 Hz, 5′-H), 3.87 (1 H, d, J = 5.0 Hz, 2-H), 3.86–4.04 (4 H, m, 11-H, 4′-H, 6′-H, and 13′-H), 3.99, 4.26 (1 H each, AB pattern, J = 12.7 Hz, 15-H), 5.57 (1 H, d, J = 6.0 Hz, 10-H), 5.76 (1 H, d, J = 11.2 Hz, 10′-H), 5.89 (1 H, dd, J = 3.0 and 15.5 Hz, 7′-H), 6.17 (1 H, dd overlapping with 2′, 4-H), 6.18 (1 H, br s, 2′-H), 6.57 (1 H, dd, $J_{8',9'} = J_{9',10'}$ = 11.2 Hz, 9′-H), 7.44 (1 H, dd, $J_{7',8'}$ = 15.5 Hz and $J_{8',9'}$ = 11.2 Hz, 8′-H); mass spectrum (NICI, ammonia reagent gas) calcd for $C_{29}H_{38}O_{10}$ *m/e* 546.2465, found 546.2425.

B14 triacetate: ^1H NMR δ 0.79 (3 H, s, 14-H), 1.19 (3 H, d, $J_{13',14'}$ = 6.4 Hz, 14′-H), 1.69 (3 H, s, 16-H), 2.07, 2.09, 2.14 (3 H each, s, CH_3COO), 2.00–2.15 (1 H, m, 3β-H), 2.30 (3 H, s, 12′-H), 2.46 (1 H, dd, J = 8.2 and 15.7 Hz, 3α-H), 2.82, 3.14 (1 H each, AB pattern, J = 4.0 Hz, 13-H), 3.65, 3.85 (1 H each, d of AB pattern, J = 4.3 and 9.7 Hz, 5′-H), 3.73 (1 H, d, J = 5.4 Hz, 11-H), 3.85 (1 H, d, J = 4.7 Hz, 2-H), 3.90, 4.59 (1 H each, AB pattern, J = 12.7 Hz, 15-H), 4.07 (1 H, br s, 6′-H), 4.99 (1 H, dq, $J_{6',13'}$ = 2.5 Hz and $J_{13',14'}$ = 6.4 Hz, 13′-H), 5.28 (1 H, t, J = 4.3 Hz, 4′-H), 5.30 (1 H, dd, J = 4.6 and 10.8 Hz, 8-H), 5.60 (1 H, d, J = 5.4 Hz, 10-H), 5.74 (1 H, d, J = 11.3 Hz, 10′-H), 5.87 (1 H, dd, J = 3.4 and 15.2 Hz, 7′-H), 5.93 (1 H, s, 2′-H), 6.53 (1 H, dd, $J_{8',9'} = J_{9',10'}$ = 11.3 Hz, 9′-H), 7.61 (1 H, dd, J = 11.3 and 15.2 Hz, 8′-H); mass spectrum (NICI, ammonia reagent gas) calcd for $C_{35}H_{44}O_{13}$ *m/e* 672.2782, found 672.2745.

Fraction B2 was crystallized from CH_2Cl_2/ether and recrystallized from ethyl acetate to give 83 mg of baccharinoid B13 (**20**): mp 218–219 °C; $[\alpha]^{25}_D$ +130 ∓ 1.0 (*c* 0.74, MeOH); UV λ_{max} 220, 262 nm; IR (CH_2Cl_2) 3600, 2880, 1720, 1180 cm^{-1}; ^1H NMR δ 0.76 (3 H, s, 14-H), 1.21 (3 H, d, J = 6.4 Hz, 14′-H), 1.81 (3 H, s, 16-H), 2.05–2.15 (1 H, m, 3β-H), 2.22 (3 H, d, J = 1.2 Hz, 12′-H), 2.51 (1 H, dd, J = 8.3 and 15.7 Hz, 3α-H), 2.83, 3.14 (1 H each, AB pattern, J = 4.0 Hz, 13-H), 3.62–3.79 (3 H, m, 13′-H, 5′B-H, and 6′-H), 3.85 (1 H, d, J = 5.2 Hz, 2-H), 3.90–3.96 (2 H, m, 11-H and 5′A-H), 3.93, 4.23 (1 H each, AB pattern, J = 12.5 Hz, 15-H), 4.05 (1 H, br s, 8-H), 4.37 (1 H, br s, 4′-H), 5.54 (1 H, d, J = 5.6 Hz, 10-H), 5.75 (1 H, d, J = 11.5 Hz, 10′-H), 5.85 (1 H, dd, J = 3.0 and 15.7 Hz, 7′-H), 6.21 (1 H, dd, J = 3.9 and 8.1 Hz, 4-H), 6.24 (1 H, br s, 2′-H), 6.55 (1 H, dd, $J_{8',9'} = J_{9',10'}$ = 11.5 Hz, 9′-H), 7.48 (1 H, dd, J = 11.5 and 15.7 Hz, 8′-H); mass spectrum (NICI, ammonia reagent gas) calcd for $C_{29}H_{38}O_{10}$ *m/e* 546.2465, found 546.2434.

B13 triacetate: ^1H NMR δ 0.79 (3 H, s, 14-H), 1.17 (3 H, d, J = 6.4 Hz, 14′-H), 1.70 (3 H, s, 16-H), 2.06, 2.10, 2.14 (3 H each, s, CH_3COO), 2.30 (3 H, s, 12′-H), 2.46 (1 H, dd, J = 8.3 and 15.5 Hz, 3α-H), 2.76, 3.14 (1 H each, AB pattern, J = 4.0 Hz, 13-H), 3.72 (3 H, m, 11-H, 5B′-H, and 6′-H), 3.86 (1 H, d, J = 5.3 Hz, 2-H), 3.85, 4.63 (1 H each, AB pattern, J = 12.7 Hz, 15-H), 5.20 (1 H, dq, $J_{6',13'} = J_{13',14'}$ = 6.4 Hz, 13′-H), 5.27 (1 H, t, J = 4.1 Hz, 4′-H), 5.32 (1 H, dd, J = 5.6 and 10.4 Hz, 8-H), 5.60 (1 H, d, J = 5.5 Hz, 10-H), 5.73 (1 H, d, J = 11.3 Hz, 10′-H), 5.82–5.92 (3 H, m, 7′-H, 2′-H, and 4-H), 6.56 (1 H, dd, $J_{8',9'} = J_{9',10'}$ = 11.3 Hz, 9′-H), 7.40 (1 H, dd, J = 11.3 and 15.5 Hz, 8′-H); mass spectrum (NICI, ammonia reagent gas) calcd for $C_{35}H_{44}O_{13}$ *m/e* 672.2782, found 672.2784.

Fraction C was injected onto a semiprep HPLC column (NH_2-silica gel column, 18 injections) with 2% MeOH in CH_2Cl_2 as eluent to give as colorless glasses 38 mg of baccharinoid B23 (**22**) and 42 mg of baccharinoid B24 (**23**).

B23 (22): $[\alpha]^{25}_D$ +113.5 ± 0.5 (*c* 0.9, MeOH); UV λ_{max} 261 nm; IR (CH_2Cl_2) 3600, 2870, 1715, 1730, 1175 cm^{-1}; ^1H NMR δ 0.77 (3 H, s, 14-H), 1.00 (3 H, d, J = 6.4 Hz, 12′-H), 1.21 (3 H, d, J = 6.4 Hz, 14′-H), 2.10–2.25 (3 H, m, 3β-H and 2′-H), 2.40–2.53 (2 H, m, 3α-H and 3′-H), 2.82, 3.12 (1 H each, AB pattern, J = 4.0 Hz, 13-H), 3.60–3.75 (4 H, m, 4′-H, 5′-H, and 13′-H), 3.73 (2 H, s, 16-H), 3.84 (1 H, d, J = 4.8 Hz, 2-H), 4.06 (1 H, br s, 6′-H), 3.92, 4.67 (1 H each, AB pattern, J = 12.5 Hz, 15-H), 5.79 (1 H, d, J = 11.3 Hz, 10′-H), 5.77–5.85 (2 H, m, 10-H and 4-H), 6.06 (1 H, dd, J = 3.3 and 15.7 Hz, 7′-H), 6.68 (1 H, dd, $J_{8',9'} = J_{9',10'}$ = 11.3, 9′-H), 7.81 (1 H, dd, J = 11.4 and 15.7 Hz, 8′-H); mass spectrum (NICI, ammonia reagent gas) calcd for $C_{29}H_{40}O_{10}$ *m/e* 548.2622, found 548.2572.

B23 triacetate: ^1H NMR δ 0.80 (3 H, s, 14-H), 1.06 (3 H, d, J = 6.8 Hz, 12′-H), 1.18 (3 H, d, J = 6.5 Hz, 14′-H), 2.03, 2.08, 2.10 (3 H each, s, CH_3COO), 2.05–2.25 (1 H, m, 3β-H), 2.15–2.40 (3 H, m, 2′-H and 3′-H), 2.47 (1 H, dd, J = 8.1 and 15.2 Hz, 3α-H), 2.82, 3.12 (1 H each, AB pattern, J = 4.0 Hz, 13-H), 3.50, 3.72 (1 H each, d of AB pattern, J = 4.7 and 10.3 Hz, 5′-H), 3.67 (1 H, d, J = 5.3 Hz, 11-H), 3.85 (1 H, d, J = 4.9 Hz, 2-H), 3.97–4.03 (1 H, m, 6′-H), 4.01, 4.53 (1 H each, AB pattern, J = 12.3 Hz, 15-H), 4.49 (2 H, s, 16-H), 4.85 (1 H, dd, J = 4.7 and 10.4 Hz, 4′-H), 5.05 (1 H, dq, $J_{6',13'} = J_{13',14'}$ = 6.5 Hz, 13′-H), 5.73 (1 H, d, J = 5.3 Hz, 10-H), 5.78 (1 H, d, J = 11.3 Hz, 10′-H), 5.83 (1 H, dd, J = 4 and 8 Hz, 4-H), 5.93 (1 H, dd, J = 3.2 and 15.2 Hz, 7′-H), 6.62 (1 H, dd, $J_{8',9'} = J_{9',10'}$ = 11.3 Hz, 9′-H), 7.60 (1 H, dd, J = 11.3 and 15.2 Hz, 8′-H); mass spectrum (NICI, ammonia reagent gas) calcd for $C_{35}H_{46}O_{13}$ *m/e* 674.2938, found 674.2926.

B24 (23): $[\alpha]^{25}_D$ +90.8 ± 0.4 (*c* 1.36, MeOH); UV λ_{max} 260 nm; IR (CH_2Cl_2) 360, 2875, 1720, 1715, 1180 cm^{-1}; ^1H NMR δ 0.79 (3 H, s, 14-H), 1.01 (3 H, d, J = 6.5 Hz, 12′-H), 1.17 (3 H, d, J = 6.4 Hz, 14′-H), 2.10–2.50 (5 H, m, 3-H, 2′-H, and 3′-H), 2.82, 3.12 (1 H each, AB pattern, J = 4.0 Hz, 13-H), 3.62–3.74 (4 H, m, 11-H, 5′-H, and 13′-H), 3.72 (2 H, s, 16-H), 3.84 (1 H, d, J = 4.8 Hz, 2-H), 4.04 (1 H, m, 6′-H), 3.94, 4.63 (1 H each, AB pattern, J = 12.4 Hz, 15-H), 5.69 (1 H, d, J = 5.3 Hz, 10-H), 5.78 (1 H, d, J = 11.3 Hz, 10′-H), 5.84 (1 H, dd, J = 4.5 and 8.2 Hz, 4-H), 6.06 (1 H, dd, J = 3.0 and 15.6 Hz, 7′-H), 6.69 (1 H, dd, $J_{8',9'} = J_{9',10'}$ = 11.3 Hz, 9′-H), 7.79 (1 H, dd, J = 11.3 and 15.6 Hz, 8′-H); mass spectrum (NICI, ammonia reagent gas) calcd for $C_{29}H_{40}O_{10}$ *m/e* 548.2621, found 548.2581.

B24 triacetate: ^1H NMR δ 0.80 (3 H, s, 14-H), 1.07 (3 H, d, J = 6.8 Hz, 12′-H), 1.21 (3 H, d, J = 6.5 Hz, 14′-H), 2.06, 2.08, 2.10 (3 H each, s, CH_3COO), 2.10–2.25 (2 H, m, 3β-H, 2′B-H), 2.35–2.53 (2 H, m, 3α-H, 2′A-H), 2.65–2.80 (1 H, m, 3′-H), 2.82, 3.12 (1 H each, AB pattern, J = 4.0 Hz, 13-H), 3.57, 3.69 (1 H each, d of AB pattern, J = 4.7 and 10.3 Hz, 5′-H), 3.65 (1 H, d, J = 5.0 Hz, 11-H), 3.85 (1 H, d, J = 4.9 Hz, 2-H), 4.03 (1 H, m, 6′-H), 3.99, 4.57 (1 H each, AB pattern, J = 12.5 Hz, 15-H), 4.49 (2 H, s, 16-H), 4.84 (1 H, dd, J = 4.7 and 10.6 Hz, 4′-H), 4.86 (1 H, dq, $J_{6',13'} = J_{13',14'}$ = 6.5 Hz, 13′-H), 5.73 (1 H, d, J = 5.0 Hz, 10-H), 5.79 (1 H, d, J = 11.4 Hz, 10′-H), 4.82 (1 H, dd, J = 4.5 and 8.1 Hz, 4-H), 5.93 (1 H, dd, J = 3.3 and 15.5 Hz, 7′-H), 6.60 (1 H, dd, $J_{8',9'} = J_{9',10'}$ = 11.4 Hz, 9′-H), 7.58 (1 H, dd, J = 11.4 and 15.5 Hz, 8′-H); mass spectrum (NICI, ammonia reagent gas) calcd for $C_{35}H_{46}O_{13}$ *m/e* 674.2938, found 674.2934.

Isolation of Baccharinoid B16 (26). After NaOH treatment, fraction IX-E (Scheme III, 70 g) was subjected to column chromatography (MPLC) on silica (190 g) with 30–100% ethyl acetate in hexane as eluent to give two fractions containing trichothecenes: A (35 g) and B (10 g). Fraction A was further fractionated on the Chromatotron (4-mm SiO_2 plate, 70% ethyl acetate/hexane) to give two compounds whose properties were identical to those of trichoverrols A and B (**19**).[14]

Fraction B was purified on the Chromatotron (SiO_2, 60% ethyl acetate/hexane) to give a fraction, which was crystallized from CH_2Cl_2/ether and recrystallized from ethanol/hexane to give 350 mg of baccharinoid B16 (**26**): mp 160–161 °C $[\alpha]^{25}_D$ +63.5 ± 0.5 (*c* 0.47, MeOH); UV λ_{max} 220, 260 nm; IR (CH_2Cl_2) 3600, 2860, 1710, 1180 cm^{-1}; ^1H NMR δ 0.78 (3 H, s, 14-H), 1.16 (3 H, d, J = 6.4 Hz, 14′-H), 2.15–2.00 (1 H, m, 3β-H), 2.22 (3 H, d, J = 1.0 Hz, 12′-H), 2.54 (1 H, dd, J = 8.2 and 15.5 Hz, 3α-H), 2.81, 3.12 (1 H each, AB pattern, J_{AB} = 4.0 Hz, 13-H), 3.62, 3.76 (1 H each, d of AB pattern, J = 3.7 and 9.1 Hz, 5′-H), 3.63 (1 H, d, overlapping with 5′A, 11-H), 3.84 (1 H, d, J = 4.9 Hz, 2-H), 3.96–4.07 (1 H, m, 6′-H), 4.01 (2 H, br s, 16-H), 4.26 (1 H, br s, 4′-H), 4.04, 4.29 (1 H each, AB pattern, J_{AB} = 12.5 Hz, 15-H), 5.74 (1 H, d, $J_{9',10'}$ = 11.4 Hz, 10′-H), 5.82 (1 H, d, J = 5.4 Hz, 10-H), 5.86 (1 H, dd, $J_{6',7'}$ = 2.8 Hz and $J_{7',8'}$ = 15.8 Hz, 7′-H), 6.08 (1 H, br s, 2′-H), 6.55 (1 H, dd, $J_{8',9'} = J_{9',10'}$ = 11.4 Hz, 9′-H), 7.38 (1 H, dd, $J_{7',8'}$ = 15.8 Hz and $J_{8',9'}$ = 11.4 Hz, 8′-H); mass spectrum (NICI, ammonia reagent gas) calcd for $C_{29}H_{38}O_{10}$ *m/e* 546.2465, found 546.2465.

B16 Triacetate: ^{1}H NMR δ 0.82 (3 H, s, 14-H), 1.20 (3 H, d, J = 6.6 Hz, 14'-H), 2.08, 2.08, 2.14 (3 H each, s, CH_3COO), 2.20–2.32 (1 H, m, 3β-H), 2.30 (3 H, d, J = 1.1 Hz, 12'-H), 2.48 (1 H, dd, J = 8.3 and 15.6 Hz, 3α-H), 2.83, 3.12 (1 H each, AB pattern, J_{AB} = 4.0 Hz, 13-H), 3.73 (1 H, d, J = 5.2 Hz, 11-H), 3.65, 3.87 (1 H each, d of AB pattern, J = 4.4 and 9.6 Hz, 5'-H), 3.82–3.91 (2 H, m, 2-H and 6'-H), 3.87, 4.59 (1 H each, AB pattern, J_{AB} = 12.7 Hz, 15-H), 4.48 (2 H, br s, 16-H), 5.2 (1 H, dq, $J_{6',13'}$ = 2.7 Hz and $J_{13',14'}$ = 6.6 Hz, 13'-H), 5.27 (1 H, t, J = 4.4 Hz, 4'-H), 5.75 (1 H, d, J = 5.2 Hz, 10-H), 5.76 (1 H, d, J = 11.2 Hz, 10'-H), ~5.89 (1 H, dd, $J_{6',7'}$ = 3.0 Hz and $J_{7',8'}$ = 15.3 Hz, 7'-H), 5.91 (1 H, br s, 2'-H), 6.54 (1 H, dd, $J_{8',9'}$ = $J_{9',10'}$ = 11.2 Hz, 9'-H), 7.41 (1 H, dd, J = 15.3 and 11.2 Hz, 8'-H).

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Facile Aryl Ether Hydrolysis: Kinetics and Mechanism of 9-Anthryl Ether Cleavage in Aqueous Solution

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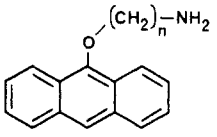
The hydrolysis rates of anthryl ether, 4-(9-anthroxy)butylamine (1), have been determined in aqueous solution from pH 1 to 12 and 25 to 80 °C, wherein it was found that this ether reacted significantly faster than other aryl ethers. The observed rate constants varied with pH according to eq 1 and values for k_{H^+}, k_0, k_N, and K_a

$$k_{obs} = (k_{H^+}[H^+] + k_0)[H^+]/(K_a + [H^+]) + k_N K_a/(K_a + [H^+]) \quad (1)$$

have been evaluated. At low pH, hydrolysis of 1 is characterized by general acid catalysis with carboxylic acid buffers (α = 0.61 ± 0.06) and a solvent isotope effect of 1.85 ± 0.09 at 25 °C. Further, the product deuterium isotope effect for reaction of 1 and H^+ is ~3 and, when the reaction is carried out in ^{18}O water, the anthrone reaction product contains exclusively ^{18}O. These observations show unequivocally that acid hydrolysis occurs by rate-determining proton transfer to the 10-position of the anthracene moiety, followed by rapid reaction of water at the 9-position of the oxonium ion intermediate. The acid-catalyzed hydrolysis of 1 was thus found to resemble vinyl ether hydrolysis (i.e., an A-S_E2 mechanism) instead of an A-2 mechanism shown by most other aryl ethers, such as anisole.

As part of our research into DMARD's (Disease-Modifying Anti-Rheumatic Drugs), we studied the aqueous hydrolysis of anthryl ether 1 (n = 4) and its 3- and 5-methylene analogues (shown below) and found them to be significantly more reactive than most other aryl ethers.



n = 3, 4, 5

1 [n = 4]

In acidic solution anthryl ether 1 degraded ~10^8 times faster than anisole or other phenyl ethers—a rate enhancement shown only by activated ethers such as vinyl ethers.[1-5] This huge disparity in reaction rates is not due to a substituent effect but to a change in reaction mechanism. Vinyl ethers, for example, react by rate-determining carbon protonation followed by rapid attack of water on the oxonium intermediate,[6-8] whereas aryl ethers are thought to hydrolyze in strong acids by preequilibrium protonation of oxygen followed by rate-determining S_N2 nucleophilic displacement. It is unknown whether the enhanced reactivity of anthryl ethers can be explained by a C-protonation hydrolysis mechanism since, even though aryl alkyl ethers readily undergo ring protonation and hydrogen isotope exchange in superacid media,[9,10] hydrolysis of aryl alkyl ethers by rate-determining carbon protonation is uncommon. 9-Anthryl ethers, however, may be good candidates for showing this unusual behavior. Should protonation occur at the 10-position of the anthryl moiety, benzo group formation will stabilize the anthryl oxonium intermediate and thus compensate for the loss of the anthryl 14 π-electron aromaticity.

Despite the fact that the 9-anthroxy moiety is used routinely as a useful hydroxyl-protecting group which may be cleaved only by singlet oxygen[11,12] and that the anthryl ether group is present in several investigational DMARD's,[13] surprisingly little is known about the hydrolysis of 9-anthryl ethers. To shed some light on the aqueous chemistry of anthryl ethers, the acid, neutral, and base catalyzed hydrolysis reactions of 1 were investigated by determination of reaction products, rate constants, and isotope effects.

Results and Discussion

General. Under anaerobic reaction conditions, hydrolysis of anthryl ether 1 obeyed first-order kinetics and afforded anthrone (2) as the primary reaction product from pH 1 to 12. In the presence of oxygen, however, varying

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