(11) W. E. Morgan and J. R. Van Wazer, *J. Am. Chem. Soc.*, **97**, 6347 (1975).
(12) For an example of ionic strength and countercation effects on another ^{31}P NMR parameter, see A. J. R. Costello, T. Glonek, and J. R. Van Wazer, *Inorg. Chem.*, **15**, 972 (1976), where ionic strength and countercation effects on ^{31}P chemical shifts are discussed.
(13) A curve of strikingly similar shape was obtained by T. L. James and M. Cohn, *J. Biol. Chem.*, **249**, 2599 (1974), when the negative ^{13}C NOE of the formate ion was plotted as a function of the ratio of formate concentration to creatine kinase binding site concentration.
(14) J. R. Van Wazer, *Phosphorus and Its Compounds*, Interscience Publishers, Inc., New York, 1958, Vol. 1, "Chemistry", pp. 486–491.
(15) T. Glonek *et al.*, work in progress.

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Baccharin, a Novel Potent Antileukemic Trichothecene Triepoxide from *Baccharis megapotamica*[1,2]

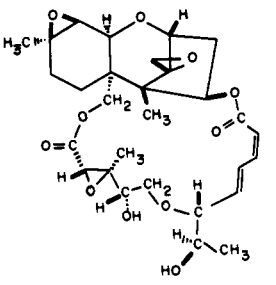
Sir:

In the course of a continuing search for tumor inhibitors from plant sources, we found that an ethanol extract of *Baccharis megapotamica* Spreng (Asteraceae)[3] showed significant inhibitory activity in vitro against cells derived from human carcinoma of the nasopharynx (KB) and in vivo against P-388 leukemia in mice.[4] We report herein the isolation and structural elucidation of one of the novel and very potent antileukemic principles, baccharin (**1**), which appears to be the first reported trichothecene triepoxide isolated from natural sources.

Fractionation of the alcohol extract, guided by a combination of P-388 in vivo assay in mice and KB testing in vitro showed that the inhibitory activity was concentrated, successively, in the ethyl acetate layer of an ethyl acetate–water partition and in the methanol layer of a 90% aqueous methanol–petroleum ether partition. Column chromatography on alumina (activity II, III) gave several active fractions; chromatography on Silica Gel 60 of one of these fractions followed by preparative TLC on Silica Gel 60 afforded baccharin (**1**, 0.004%): mp 200–230 °C (dichloromethane–methanol); $[\alpha]^{24}D$ +41.5° (*c* 2.2, $CHCl_3$); uv_{max} (EtOH) 259 nm (ϵ 18 700); ir ($CHCl_3$) 2.72, 2.9 (broad), 5.68, 5.80, 6.08, and 6.22 μ; mass spectrum (chemical ionization: methane gas reagent) *m/e* 563.2476 ($M^+ + H$, calcd 563.2492), 409, 275, 257, 137; 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, d of d, $J_{3\alpha,4\alpha}$ = 8.8; $J_{3\alpha,3\beta}$ = 16 Hz, 3α-H), 2.75, 3.16 (each 1 H, d, J = 4.0 Hz, 13-H), 3.11 (1 H, d, $J_{10,11}$ = 5.8 Hz, 10-H), 3.37 (1 H, s, 2′-H), 4.24, 4.42 (each 1 H, d, J = 12.2 Hz, 15-H), ≈5.80 (1 H, 4-H), 5.82 (1 H, d, $J_{9',10'}$ = 11 Hz, 10′-H), 5.98 (1 H, d of d, $J_{6',7'}$ = 2; $J_{7',8'}$ = 15.5 Hz, 7′-H), 6.60 (1 H, d of d, $J_{8',9'} = J_{9',10'}$ = 11 Hz, 9′-H), 7.48 (1 H, d of d, $J_{7',8'}$ = 15.5; $J_{8',9'}$ = 11 Hz, 8′-H).

Treatment of baccharin (**1**) at room temperature with acetic anhydride in pyridine gave baccharin diacetate, mp 254–256 °C; uv_{max} (EtOH) 262 nm (21 000); ir ($CHCl_3$) 5.75 (broad with shoulders), 6.10 and 6.25 μ; mass spectrum (chemical ionization: methane gas reagent) *m/e* 647.2692 ($M^+ + H$, calcd 647.2704); NMR ($CDCl_3$) δ 0.75 (3 H, s, 14-H), 1.24 (3 H, d, J = 6.6 Hz, 14′-H), 1.36 (3 H, s, 16-H), 1.74 (3 H, s, 12′-H), 2.03, 2.16 (each 3 H, s, $-COCH_3$), 2.46 (1 H, d of d, $J_{3\alpha,4\alpha}$ = 8.0; $J_{3\alpha,3\beta}$ = 16 Hz, 3α-H), 2.76, 3.16 (each 1 H, d, J = 3.9 Hz, 13-H), 3.07 (1 H, d, $J_{10,11}$ = 5.3 Hz, 10-H), 3.37 (1 H, s, 2′-H), 4.23, 4.49 (each 1 H, d, J = 12.5 Hz, 15-H), ≈4.30 (1 H, 4′-H), 5.06 (1 H, d of q, $J_{12',13'}$ = 6.6, $J_{12',6'}$ = 4 Hz, 13′-H), ≈5.75 (1 H, 4-H), 5.80 (1 H, d, $J_{9',10'}$ = 11 Hz, 10′-H), ≈5.90 (1 H, 7′-H), 6.60 (1 H, d of d, $J_{8',9'} = J_{9',10'}$ = 11 Hz, 9′-H), 7.47 (1 H, d of d, $J_{7',8'}$ = 15.5; $J_{8',9'}$ = 11 Hz, 8′-H).

Inspection of the above spectral data for baccharin (**1**) and its diacetate suggested that these compounds were related to the roridins[5] whose structures are distinguished by a 12,13-epoxytrichothecene central ring system which is spanned by a dienic macrolide ester side chain. However, careful comparison of the spectral data for **1** and its diacetate (as well as the spectral data for a number of structurally similar compounds that have been isolated from *B. megapotamica*) with the data published for the roridins[5,6] clearly showed that **1** (and other so far isolated active principles) is more highly oxygenated than the known roridins.[7]



1

The chemical structure and molecular stereochemistry of **1** were determined by a direct single-crystal x-ray analysis using a crystal obtained by slow evaporation of solvent from a solution of **1** in dichloromethane–methanol. Crystals of **1** conform to space group $P2_12_12_1$ with a = 10.389 (1) Å, b = 30.160 (2) Å, c = 10.172 (1) Å, and Z = 4. Intensity measurements were made by diffractometry with Cu Kα radiation made monochromatic by Bragg reflection from a highly oriented graphite crystal. Within a single octant of reciprocal space, surveyed to sin θ/λ 0.562, scattered intensity significantly above background [$I > 3\sigma(I)$] was measured by scintillation counting at 2471 of 2726 locations.

The structure was solved by application of the program MULTAN[9] and refined by difference Fourier and least-squares method to R = 0.07 for the significant reflections. C and O atoms were refined using anisotropic thermal parameters, and all hydrogen atoms in the molecule, with the exception of those associated with the hydroxy groups, were clearly identifiable from different maps, and fixed contributions for them were included in the least-squares calculations. Loosely bound water of solvation is found at four sites in the asymmetric unit with one site fully and the others partially occupied.

A view of the molecular structure of **1** as found in the crystal is shown in Figure 1. Although the absolute configuration of **1** has not been established by the analysis, the figure is drawn to conform to the absolute stereochemistry derived from the x-ray analysis of the *p*-iodobenzenesulfonate of verrucarin A.[10] The 12,13-epoxytricothecene structure is confirmed with epoxide functionalities found also at C(9)–C(10) and C(2′)–C(3′), a hydroxy group at C(4′), and a hydroxyethyl group at C(6′). In the central nucleus, the six-membered oxa-ring B adopts a chair conformation, the five-membered ring C an envelope form with the flap at C(12). The presence of the 9,10 epoxide unit, which acts stereochemically as a double bond, leads to a 1,2-diplanar conformation for the six-membered A ring typical of substituted cyclohexenes.

Note should be taken that isolation of **1** and related compounds from *B. megapotamica* constitutes the first known case of the appearance of 12,13-epoxytrichothecenes in higher plants; all previous isolations of such compounds have been from various species of fungi. At the moment, we cannot exclude the possibility that these compounds result from fungal

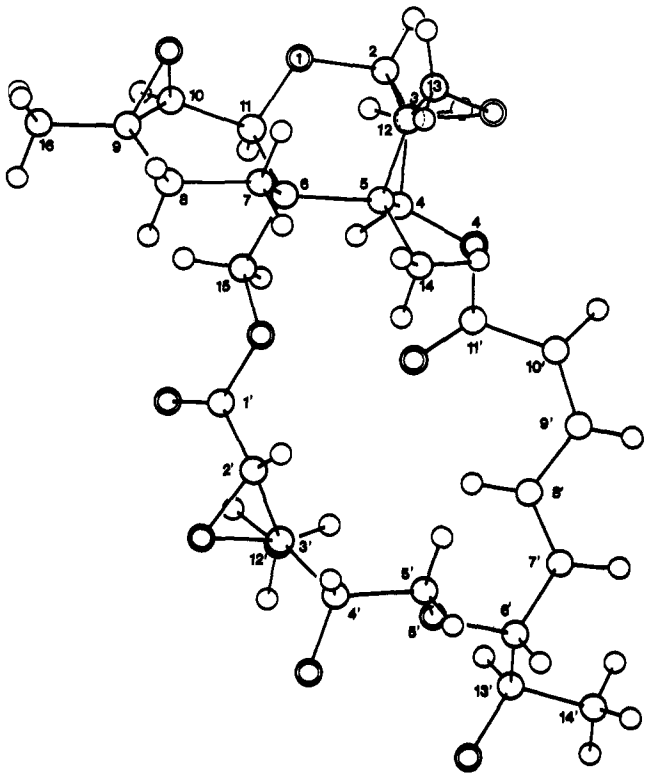


Figure 1. Diagrammatic representation of the structure of baccharin as found in the crystal. Carbon atoms are represented by the larger single circles, oxygen by double circles, and hydrogen by the smaller circles. Hydrogen atoms not located, at the O(4′) and O(13′) hydroxy groups, are omitted.

contamination; however, inspection of the plant material does not reveal any obvious contamination, and the baccharins have been isolated from two separate collections of plant material. Furthermore, the compounds are estimated to comprise ca. 0.02% of the dry plant material, a rather high percentage for such a putative contamination. What does seem a distinct possibility is that the baccharins may be plant-altered fungal metabolites.

References and Notes

(1) Tumor Inhibitors. 119. Part 118: S. M. Kupchan and D. R. Streelman, *J. Org. Chem.*, in press.

(2) This investigation was supported by research grants from the National Cancer Institute (N.C.I.) (CA-11718, CA-11760, and CA-12059) and the American Cancer Society (CI-102K), and contracts with the Division of Cancer Treatment, N.C.I., National Institutes of Health (NO1-CM-12099 and NO1-CM-67002).

(3) 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., U.S. Department of Agriculture, Baltimore, Md., in accordance with the program developed by the National Cancer Institute.

(4) 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). Baccharin showed optimal T/C values of ca. 200 at dose levels of 1.25–5.0 mg/kg. The cytotoxicity (ED_{50}) against KB cell cultures of 1 and the other related baccharins is ca. 10^{-3}–10^{-4} μg/ml. The cytotoxicity of 1 is in a range typical for trichothecenes, but the antileukemic activity (PS) of 1 exceeds substantially those of reported trichothecenes.[6]

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

(6) For an excellent review of the chemical and spectral properties of 12,13-epoxytrichothecenes, see J. R. Bamburg and F. M. Strong in "Microbial Toxins", Vol. 7, S. Kadis, A. Cieglek, and S. J. Ajl, Ed., Academic Press, New York, N.Y., 1971, pp 207–292.

(7) Baccharin (1) is the only active principle isolated from *B. megapotamica* in which the central 12,13-epoxytrichothecene 9,10 double bond is epoxidized. The remaining active principles (to date, six) all give the same central ring system upon base hydrolysis: 4β,8α,15-trihydroxy-12,13-epoxytrichothecene, a heretofore unknown trichothecene. There are no reports of naturally occurring trichothecenes with an epoxide at the 9,10 position, although peracid oxidation of the 9,10 double bond in roridin A[8] gives as the principal product a β-epoxide having the same configuration at C9,C10 as in 1.

(8) R. Achini and Ch. Tamm, *Helv. Chim. Acta*, **51**, 1712 (1968).

(9) G. Germain, P. Main, and M. M. Woolfson, *Acta Crystallogr., Sect. A*, **27**, 368 (1971).

(10) A. T. McPhail and G. A. Sim, *J. Chem. Soc. C*, 1394 (1966).

(11) Visiting Scholar, 1975–1976; on sabbatical leave, University of Maryland, College Park, Md. Receipt of a National Research Service Award (1F32 CA05368-01) from the National Institutes of Health is gratefully acknowledged.

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$[Mo_3(OAc)_6(CH_3CH_2O)_2(H_2O)_3]^{2+}$ and Other New Products of the Reaction between Molybdenum Hexacarbonyl and Acetic Acid

Sir:

The preparation of dimolybdenum tetraacetate $Mo_2(OAc)_4$ by refluxing $Mo(CO)_6$ in an acetic acid–acetic anhydride mixture[1] was characterized by a noticeably low yield.[2] The dark brown solution from which the crystals of the tetraacetate had been removed contained the greater part of the molybdenum products of the reaction. These products were separated by ion exchange chromatography. The solution was absorbed on a cation exchange column (Dowex 50 X2) and eluted by 0.1 M CF_3SO_3H. This elution removed a green-brown molybdenum ion (I) with an absorption maximum at 595 nm. The elution behavior of this species indicated an ionic charge of 1+. Several green bands of species with higher ionic charges remained on the column. All these products were also obtained when acetic anhydride was omitted from the reaction mixture; i.e., they were produced by the reaction of $Mo(CO)_6$ and acetic acid.

The green-brown solution of I was oxidized by permanganate to a deep red solution which was absorbed on a cation exchange column. Elution with 0.1 M acid removed a brown band of the remaining unreacted ion I. Elution with 0.5 M acid removed a red molybdenum ion II. The spectrum of II is presented in Figure 1.

When trifluoromethylsulfonic acid was used for elution of II, spontaneous crystallization took place in the eluted solution. The red crystals were filtered, rinsed with acetone and ether, and dried under vacuum. Elemental analysis[3] suggested the formula $Mo_3(OAc)_6(CH_3CH_2O)_2(H_2O)_3(CF_3SO_3)_2$. Crystallization also occurred when the ion II was eluted with perchloric acid and *p*-toluenesulfonic acid.[4] The trifluoromethylsulfonate salt was subjected to x-ray diffraction structure determination.[5,6] The structure of the complex is shown in Figures 2 and 3.

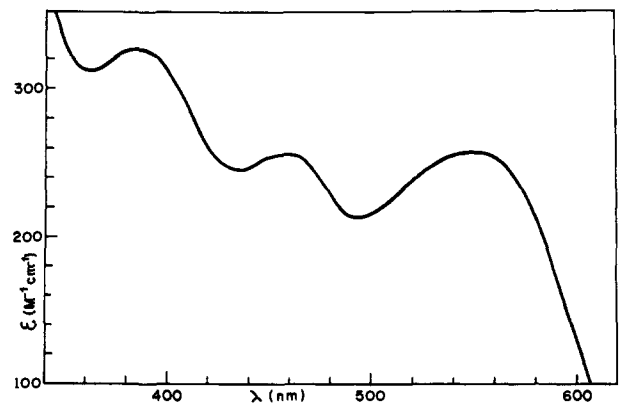

Figure 1. Absorption spectrum of $[Mo_3(OAc)_6(CH_3CH_2O)_2(H_2O)_3]^{2+}$.