Journal of Natural Products
Vol. 51, No. 5, pp. 981-984, Sep-Oct 1988

BRIANTHEIN V, A NEW CYTOTOXIC AND ANTIVIRAL DITERPENE ISOLATED FROM *BRIAREUM ASBESTINUM*

STEPHEN J. COVAL, SUE CROSS,

Harbor Branch Oceanographic Institution–SeaPharm Project, Fort Pierce, Florida 33450

GERALD BERNARDINELLI, and CHARLES W. JEFFORD*

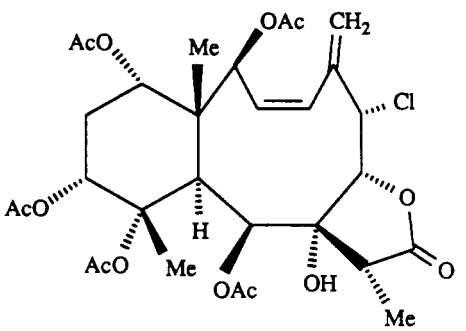
Department of Organic Chemistry and Laboratory of Crystallography, University of Geneva, 1211 Geneva 4, Switzerland

The gorgonian coral *Briareum asbestinum* (Pallas) (Briareidae), occurring in Caribbean waters, contains diterpenes of two skeletal types, the asbestinins and the briareins, some of which are endowed with biological activity (1,2). Several chlorinated briareins have also been isolated, and the absolute structure of one of them, briarein A [**1**], a pentaacetate, has been determined by X-ray (3). Subsequently, a related species *Briareum polyanthes*, found off Bermuda, was examined. Three metabolites possessing the epoxybriarein structure, but designated as briantheins X [**2**], Y [**3**], and Z [**4**] were identified (4,5). [The structure of Brianthein W, a less functionalized nonchlorinated briarein, has been recently determined (6).]

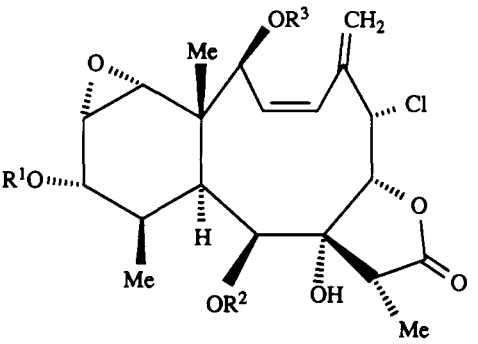
In the course of our search for biologically active compounds from marine sources, we had occasion to investigate a specimen of *B. asbestinum* collected near Sandy Cay, Bahamas, at a depth of 2 m. A voucher specimen is stored at HBOI, Seapharm No. 2-VI-84-2-6. Extraction afforded a new metabolite, which we name brianthein V [**5**], together with briantheins Y and Z. We now report on the structure elucidation of **5** and the discovery that these compounds display cytotoxic and antiviral activity.

Workup of the organic extract by solvent partitioning and flash chromatography on Si gel, followed by hplc on a C_{18} column, yielded the three diterpenes **3**–**5** in a pure state. Examination of the spectral data for **3** and **4** showed them to be briantheins Y and Z (4,5).

The spectral data for **5** were consistent with a structure similar to that of briantheins Y and Z. However, compound **5** was seen to possess an extra C_2H_4 fragment relative to **3**, as indicated by its molecular formula, $C_{30}H_{41}ClO_{10}$, which was determined by hreims. The identity of the extra group was deduced by comparing the nmr spectra of **3** and **5**. While the ^{13}C spectrum of **3** shows two signals at δ 36.3 and 18.4 belonging to the butyrate group, the spectrum of **5** displays four such signals at δ 36.3,



1



2 $R^1=R^2=Ac$, $R^3=H$
3 $R^1=R^2=Ac$, $R^3=COCH_2CH_2CH_3$
4 $R^1=R^2=R^3=Ac$
5 $R^1=R^3=COCH_2CH_2CH_3$, $R^2=Ac$

35.9, 18.6, and 18.4. The ^{1}H-nmr spectrum of **5** was identical to that of **3** except for the following differences: **5** is characterized by an additional triplet at δ 0.96 ascribable to a methyl group; the integral for the multiplet at δ 1.65 is bigger; the integral for the broad triplet at δ 2.29 corresponds to four protons; and finally, the acetate methyl singlet at δ 1.96 is missing. The foregoing data indicate that **5** differs from **3** by having a butyrate instead of an acetate group. However, the spectral information is insufficient to specify the points of attachment of the acetate and butyrate substituents. Fortunately, X-ray analysis of a suitable crystal of **5** grown from a solution of MeOH furnished the absolute structure. The structure and ring conformations of **5** (Figure 1) are wholly similar to those observed for briarein A [**1**] and brianthein Y [**3**]. The cyclodecene ring in **5** adopts practically the same conformation as that displayed by brianthein Y. The main difference with briarein A centers around the C-9–C-10 and C-10–C-1 bonds owing to the fusion of the epoxide on the six-membered ring. The latter ring adopts a C_s conformation with a mirror plane passing through the C-11 and C-14 atoms. Like brianthein Y, the *trans*-fused γ-lactone entity in **5** exists as an envelope conformation with the C-8 atom pushed out-of-plane. The observed torsional angle of 57(2)° described by the C-3–C-4–C-5–C-22 atoms which comprise the endo- and exocyclic double bonds means that conjugation is prevented. Compound **5**, just like its two relatives, has longer than usual bonds attached to the quaternary C-1 atom. The butyrate substituents are affected by sizable atomic displacements that account for the relatively short bond distances observed between atoms located at the end of the chains.

It is worth noting that **5** crystallizes with one molecule of MeOH to which it is hydrogen-bonded. However, only the structure of **5** is shown as the position of the MeOH is unexceptional.

Screening for biological activity revealed that diterpenes **4** and **5** are cytotoxic, and compounds **3–5** display antiviral activity. Compounds **4** and **5** show in vitro cytotoxicity in the P388 assay at 10 and 13 μg/ml, respectively. In the in vitro mouse corona-virus assay, compounds **3**, **4**, and **5** showed viral inhibitions at 400, 80, and 50 μg/ml, respectively. Compound **4** is also active in vitro against herpes simplex-1 virus at 80 μg/ml. [These values are to be compared with the standards used: P388 (5-fluorouracil, 0.1 μg); mouse corona virus (Ribavirin, 10.0 μg) and herpes simplex-1 virus (Ara-A, 20.0 μg)]. We

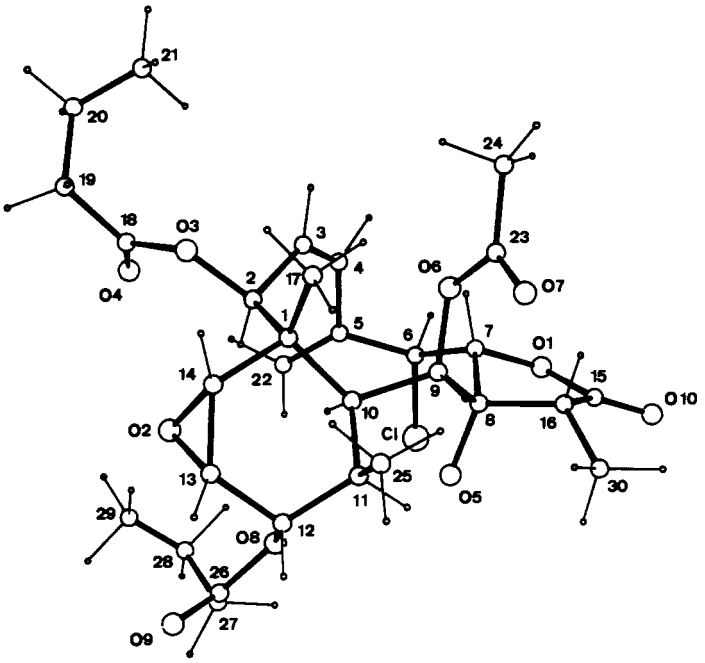


FIGURE 1. Computer-generated perspective drawing of brianthein V [**5**].

believe that these results are the first for terpenes of the briarein type (7).

EXPERIMENTAL

GENERAL.—The nmr spectra were recorded on a Bruker AM360 spectrometer. Ir spectra were recorded on a Perkin-Elmer 1310 spectrophotometer. Optical rotations were measured on a Jasco DIP-360 digital polarimeter. Mass spectra were obtained at the University of Illinois, Urbana, using a Finnigan-MAT CH5 mass spectrometer. Melting points (Gallenkamp apparatus) are uncorrected.

ISOLATION.—The freeze-dried coral (109 g) was extracted repeatedly at room temperature with MeOH. The extracts were concentrated and partitioned between MeOH-H_2O (9:1) and hexane. After removal of hexane, the aqueous MeOH fraction was diluted with H_2O to a 6:4 ratio. The resulting solution was partitioned with CH_2Cl_2. Evaporation yielded a residue (993 mg). A portion (407 mg) was subjected to vacuum flash chromatography on a silica column (2.5 × 6 cm) with hexane-EtOAc (7:3) as eluent (8). Seven 100-ml fractions were collected. The second fraction (98 mg) was subjected to hplc on a C_{18} column with MeOH-H_2O (7:3) as eluent to yield **4** (23 mg) and **6** (15 mg). Fractions 3–6 were combined. The resulting residue (55 mg) was subjected to hplc on a C_{18} column with MeOH-H_2O (3:1) as eluent and gave **5** (3 mg).

BRIANTHEIN V [**5**].—Pale yellow needles, mp 222°; $[\alpha]^{20}D$ −57° (c = 1.2, $CHCl_3$); hreims *m/z* 596.2393 for $C_{30}H_{41}ClO_{10}$ (Δ −0.5 mmμ); ir (KBr) 3490 br, 2980, 1780, 1720, 1370, 1230, 1180 cm^{-1}; ^1H nmr (360 MHz in $CDCl_3$) δ 6.32 (br s, 1H), 6.19 (d, J = 9.2, 1H), 5.98 (d, J = 2.6, 1H), 5.90 (dd, J = 1.0, 11.6, 1H), 5.59 (dd, J = 9.1, 11.6, 1H), 5.25 (br d, J = 2.6, 1H), 5.23 (d, J = 8, 1H), 4.91 (d, J = 3.5, 1H), 4.69 (dd, J = 2.7, 5.5, 1H), 3.55 (dd, J = 3.4, 5.3, 1H), 2.93 (d, J = 3.3, 1H), 2.25–2.35 (m, 8H), 2.14 (s, 3H), 1.65 (m, 4H), 1.14 (d, J = 7.0, 3H), 1.08 (s, 3H), 1.05 (d, J = 7.4, 3H), 0.96 (t, J = 7.4, 3H), 0.93 (t, J = 7.4, 3H); ^{13}C nmr (90 MHz in $CDCl_3$) δ 174.4 (s), 172.8 (s), 172.5 (s), 170.0 (s), 136.6 (s), 131.0 (d), 127.8 (d), 119.0 (t), 84.5 (s), 77.6 (d), 75.5 (d), 69.3 (d), 69.2 (d), 62.4 (d), 62.0 (d), 52.8 (d), 44.9 (d), 40.8 (s), 37.0 (d), 36.3 (t), 35.9 (t), 32.9 (d), 21.9 (q), 18.6 (t), 18.4 (t), 15.8 (q), 13.7 (q), 13.6 (q), 12.7 (q), 6.2 (q).

CRYSTALLOGRAPHIC DATA AND STRUCTURE REFINEMENT OF BRIANTHEIN V [**5**].—$C_{30}H_{41}O_{10}Cl$/MeOH, mol wt = 597.2/32.0 crystallizes in orthorhombic system, space group $P2_12_12_1$, a = 10.961(2), b = 16.617(4), c = 17.595(3) Å, Z = 4, d_c = 1.304 g/cm^3, μ = 0.172 mm^{-1}, Fooo = 1344. The diffracted intensities were measured at room temperature on an automatic four circle PW1100 diffractometer operating with graphite monochromated MoKα radiation. Data collection: sin θ/λ <0.482; h = 0.10;

$k = 0.15$; $l = 0.16$ and all antireflections of these, $\omega/2\theta$ scans, no absorption correction. Of the 2961 measured reflections, 2093 were considered as observed ($|Fo| > 4\sigma(Fo)$ and $|Fo| > 8$) and used in structure refinement. The structure was solved by direct methods (MULTAN-80) (9) and refined by full-matrix least-squares analysis. Atomic scattering factors and anomalous-dispersion terms (for the Cl atom) were used as tabulated (10). The final R factor was 0.095 ($\omega R = 0.095$, $\omega = 1$). All calculations were performed with a local version of XRAY-76 (11). The absolute configuration of the molecule was confirmed by refinement of the absolute structure parameter: [$x = 0.33(43)$] (12). As the butyrate substituents undergo substantial atomic displacements, the C-20, C-21, C-28, and C-29 atoms were refined with isotropic displacement parameters. All coordinates of the H atoms were calculated, except for those of the two hydroxyl groups for which the hydrogen atoms were not observed.[1]

LITERATURE CITED

1. D.B. Stierle, B. Carté, D.J. Faulkner, B. Tagle, and J. Clardy, *J. Am. Chem. Soc.*, **102**, 5088 (1980).
2. S.J. Selover, P. Crews, B. Tagle, and J. Clardy, *J. Org. Chem.*, **46**, 964 (1981).
3. J.E. Burks, D. van der Helm, C.Y. Chang, and L.S. Ciereszko, *Acta Crystallogr.*, **B33**, 704 (1977).
4. S.H. Grode, T.R. James, J.H. Cardellina, II, and K.D. Onan, *J. Org. Chem.*, **48**, 5203 (1983).
5. S.H. Grode, T.R. James, and J.H. Cardellina, II, *Tetrahedron Lett.*, 691 (1983).
6. J.H. Cardellina, II, T.R. James, Jr., M.H.M. Chen, and J. Clardy, *J. Org. Chem.*, **49**, 3398 (1984).
7. P.A. Keifer, K.L. Rinehart, Jr., and I.R. Hooper, *J. Org. Chem.*, **51**, 4450 (1986).
8. W.C. Still, M. Kahn, and A. Mitra, *J. Org. Chem.*, **43**, 2923 (1978).
9. P. Main, S.J. Fiske, S.E. Hull, L. Lessinger, G. Germain, J.-P. Declercq, and M.M. Woolfson, "A System of Computer Programs for the Automatic Solution of Crystal Structures from X-Ray Diffraction Data," Universities of York, England, and Louvain-la-Neuve, Belgium, 1980.
10. "International Tables for X-Ray Crystallography," Vol. IV, Kynoch Press, Birmingham, England, 1974.
11. J.M. Stewart, P.A. Machin, C.W. Dickinson, H.L. Ammon, H. Heck, and H.D. Flack, "The XRAY76 System," Technical Report TR-446, Computer Science Center, University of Maryland, College Park, Maryland, 1976.
12. G. Bernardinelli and H.D. Flack, *Acta Crystallogr.*, **A41**, 500 (1985).

Received 25 January 1988

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