Antitumor Plants. 12.[1,2] Further Sesquiterpenoid Constituents of *Lychnophora affinis* Gardn. (Compositae). X-ray Structure Analysis of Lychnophorolide A

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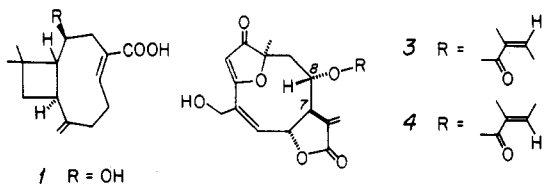
The caryophyllene derivative lychnophoric acid (2) and several eremantholides have been isolated from the cytotoxic extracts of *L. affinis* as well as two germacranolides, lychnophorolides A and B, having both α-methylene γ-lactone and 3(2*H*)-furanone electrophilic functions. The structure of lychnophorolide A has been shown to be **3** by X-ray analysis, and lychnophorolide B is assigned structure **4**. Comparison of bond lengths and angles from the crystal structure determination of lychnophorolide A and the previously determined eremantholides A and B shows a remarkable conformational similarity in the cyclodecadienone rings of these compounds.

Previous publications in this series have described some of our work on the constituents of *Lychnophora affinis* Gardn. (Compositae), especially the petroleum ether-soluble constituents,[4] the flavonoids,[5] and the novel caryophyllene derivative lychnopholic acid (**1**).[6] Our interest



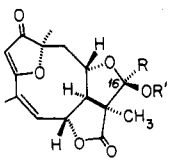
1 R = OH
2 R = H

in this plant arose from the activity of its extracts in the KB and PS assay systems;[7] although initial aqueous ethanol extracts were only marginally active (ED_{50} = 50 μg/mL vs. KB; T/C = 136% vs. PS), partition according to established procedures[6] gave a fraction soluble in methanol/water (90:10), having activity vs. KB at 15 μg/mL, and countercurrent distribution of this latter material furnished fractions having activity vs. KB at 0.2–0.4 μg/mL.

Despite the significant concentration of activity in some of the subfractions from countercurrent distribution, this method was found to offer no ultimate advantage in the fractionation procedure, since the extractives were a highly complex mixture containing many types of compounds. We therefore used conventional extraction and chromatographic techniques for the main isolations both of active materials (KB assay monitoring) and inactive congeners of chemical interest.

Early in the investigation of the petroleum ether soluble constituents, we noted the presence of acidic materials in substantial amount. In addition to octacosanoic, triacontanoic, and hentriacontanoic acids, a new compound, $C_{15}H_{22}O_2$, was obtained, which was named lychnophoric acid. Infrared and ^1H NMR spectra established the presence of a terminal methylene group and an α,β-unsaturated carbonyl group; lychnophoric acid is therefore bicyclic. Two 3H singlets near δ 1.0 suggested a *gem*-dimethyl group. Attempted dehydrogenation with 30% Pd/C[8] yielded no naphthalenic or azulenic materials, strongly suggesting a structure lacking five-, six-, or seven-membered carbon rings. This implication was substantiated when the ^{13}C NMR spectrum was seen to be very similar to that of lychnopholic acid[6] (see also the Experimental Section); the data taken together establish lychnophoric acid as **2**. Lychnophoric acid was also abundant in the methanol/water (90:10) fraction. It is probably identical with a compound obtained recently by Bohlmann et al. from *L. salicifolia* Mart.[9]

Extensive chromatography of the fraction soluble in methanol/water (90:10) gave several compounds. The first was revealed, by an X-ray structure analysis, to have structure **3**, making it the 8-epimer of budlein A.[10] This compound, which was named lychnophorolide A, showed ED_{50} vs. KB at 0.3 μg/mL, activity an order of magnitude greater than that of eremantholide A (**5**).[11] This difference



5 R = ⟨ , R' = H
6 R = ⟨ , R' = H
7 R = ⟩ H , R' = H
8 R = ⟩ H , R' = Me
9 R = ⟩ H , R' = Et
10 R = ⟨ , R' = H

(1) For part 11 in this series, see: Raffauf, R. F.; Menachery, M. D.; Le Quesne, P. W.; Arnold, Clardy, J. *J. Org. Chem.* **1981**, *46*, 1094.
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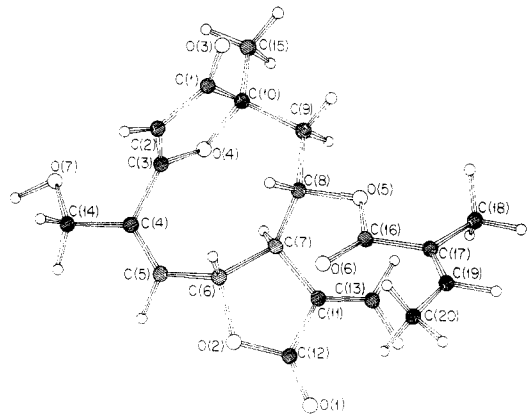


Figure 1. Computer-generated perspective drawing of lychnophorolide A.

is presumably owing to the possession by **3** of *two* electrophilic functions, the α-methylene γ-lactone and the 3(2*H*)-furanone, to which covalent binding by nucleic acids and/or enzymes can be envisaged.[11,12] An isomer, lychnophorolide B, is assigned structure **4** (a tiglate rather than an angelate side chain) on the basis of identical mass spectral base peaks at *m/e* 83 and the presence in the ^{1}H NMR spectrum of **4** of a quartet at δ 6.71, indicative of a tiglate vinyl hydrogen. The 4-deoxy 8-epimer of **4**, atripliciolide tiglate, has been identified by Bohlmann.[13]

A computer generated perspective drawing of **3** is shown in Figure 1. The X-ray experiment did not define the absolute configuration but Figure 1 was drawn to show the configuration which is in accord with the germacrane precursors from which lychnophorolide A may be regarded as having arisen.

The overall molecular conformation of **3** is very similar to those of eremantholides A (**5**) and B (**10**) even though the C(6), C(7), and C(8) substituents in the latter two form fused five-membered rings.[11] The closely similar conformations of the three cyclodecadienone rings can be illustrated by a comparison of their endocyclic torsion angles. Indeed, in this respect **3** proves to be conformationally slightly closer to either **5** or **10** than **5** is to **10**. The magnitudes of the mean differences between **3** and **5**, **3** and **10**, and **5** and **10** are 2.6°, 3.4°, and 4.3°, respectively; the respective magnitudes of the largest differences are 8.9° [C(3)–C(4)], 9.8° [C(6)–C(7)], and 10.3° [C(3)–C(4)].

The C(6),C(7) trans-fused α-methylene γ-lactone in **3** is quite planar, with the largest deviation from the best mean plane through atoms C(6), C(7), C(11), C(12), and O(2) being 0.02 Å. The signs of the O(2)–C(6)–C(7)–C(11) and C(13)–C(11)–C(12)–O(1) torsion angles are not paired;[14] however, the former is very small (–0.8°), and the latter may be affected by the proximity of O(5) [O(5)···C(11), 2.75 Å; O(5)···C(13), 2.96 Å].

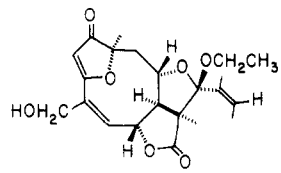
The conformation of the cyclodecadienone ring is essentially dictated by the β-side ether linkage between C(3) and C(10). Not only does this bridge require the near-planarity of atoms C(1), C(2), C(3), and C(10) but it also causes a severe transannular interaction between O(4) and H(7). The accommodation of these two strains results in an orthogonal orientation of the C(2)–C(3) and C(4)–C(5) double bonds [C(2)–C(3)–C(4)–C(5), –92.3°]. Further separation of O(4) from H(7) is effected by the adoption of an envelope form by the 3(2*H*)-furanone ring (distance from O(4) to plane of C(1), C(2), C(3), and C(10) is 0.12 Å).

In accord with what has been observed for other naturally occurring terpenoids containing a bridgehead double bond, this double bond does not appear particularly distorted.[15] The C(1)–C(2)–C(3)–O(4) torsion angle is only –5.1° and the bridgehead carbon is only slightly pyramidalized (distance from C(3) to plane of C(2), C(4), and O(4) is 0.08 Å). This lack of distortion may be due to the conjugation of the double bond with the C(1) carbonyl group.

The 3(2H)-furanone ring in **3**, while very similar to those in eremantholides A (**5**) and B (**10**), is overall flatter than either [sum of endocyclic torsion angles is 26.3° (**3**), 33.7° (**5**), and 33.6° (**10**)].

Lychnophorolide A forms a hydrogen bond (O···O separation 2.81 Å) between the O(7) hydroxyl hydrogen atom and O(3), the furenone carbonyl oxygen atom, related by a unit translation along *z*. All other intermolecular separations conform to normal van der Waals contacts.

Five eremantholides were also obtained from the fraction soluble in methanol/water (90:10). The first was the known eremantholide C (**6**),[11] and the second corresponds to the recently described compound **7**,[16] the side-chain *E* isomer of which occurs in *Eremanthus bicolor*.[9] The methyl ether **8** was characterised by a singlet signal at δ 2.90 in the ^{1}H NMR spectrum, which was assigned to the 16-methoxyl group. Recourse to Dreiding models shows that restricted rotation of the adjacent butenyl substituent leads to a general shielding of the methoxyl methyl protons which would explain the somewhat abnormal chemical shift of these protons. The mass spectrum of this compound features loss of methanol rather than OH or water from the molecular ion. Treatment of compound **8** with hydrogen chloride in tetrahydrofuran gave compound **7**. The ethyl ether **9** had IR and UV spectra identical with those of compound **8**. In the ^{1}H NMR spectrum peaks assignable to an ethoxyl group (2 H, δ 3.06–3.40; 3 H, triplet, δ 1.00) were seen, and the mass spectrum featured facile cleavage of the ethoxyl group. The fifth eremantholide, $C_{22}H_{28}O_7$, lacked in the ^{1}H NMR spectrum the typical signal for a C-4 methyl group. Instead, a broad singlet at δ 4.20, ascribable to the CH_2O protons of an allylic primary alcohol, was observed. The signal from the C-5 vinyl hydrogen appeared downfield by 0.26 ppm vs. that in compound **9**, and the side-chain vinyl proton was very slightly shifted; otherwise the spectrum was superimposable on that of compound **9**. Strucutre **11** is



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therefore proposed for this compound. It is likely that these methyl and ethyl ethers are, at least in part, artifacts of the isolation procedures.

This work, taken together with that of Bohlmann cited herein, shows the close affinities which exist between secondary metabolites of the genera *Eremanthus*, *Lychnophora*, *Piptolepis*, and *Vanillosmopsis* in the tribe

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Vernoniae. It also suggests that these genera may yet yield further new bifunctional, highly cytotoxic sesquiterpenoids.

Experimental Section[17]

Extracts and Fractions. The fraction of interest, soluble in methanol/water (90:10), was obtained as described previously.[5] It was divided into sodium bicarbonate soluble, sodium carbonate soluble, and nonacidic subfractions in the usual way.

Acidic Constituents. Chromatography of the sodium bicarbonate soluble subfraction on silica gel (W.R. Grace, 28/200 mesh) by using gradient elution (hexane–benzene–ether) yielded lychnopholic acid (1),[6,9] and lychnophoric acid (2) (data comparable with those in ref 9 except mp 119–120 °C). The ^{13}C NMR spectrum of lychnophoric acid is as follows (numbering as in ref 6; chemical shifts in parts per million downfield from Me_4Si): 52.1 (C-1), 27.4 (C-2), 23.7 (C-3), 132.2 (C-4), 144.7 (C-5), 34.0 (C-6), 28.5 (C-7), 154.4 (C-8), 111.5 (C-12), 40.3 (C-9), 40.2 (C-10), 33.3 (C-11), 22.9 (C-13), 30.0 (C-14), 173.8 (C-16).

The sodium carbonate soluble subfraction on similar chromatography yielded the flavonoid compounds previously described.[5]

Attempted Dehydrogenation of Lychnophoric Acid (2). The acid (250 mg) was mixed with 30% Pd/C (250 mg) and kept at 350–400 °C for 30 min. Extraction of the residue with hexane, chloroform, and methanol gave only minute amounts of materials, which could not be characterized.

Nonacidic Constituents. The nonacidic subfraction was chromatographed on silica gel (E. Merck, 240–400 mesh) with a 25:1 ratio of adsorbent to organic material. Gradient elution utilized hexane–benzene–ethyl acetate mixtures of increasing polarity.

The triterpenoids previously described[4] were eluted with hexane–benzene (1:1). The following compounds were obtained from more polar fractions, after subsequent preparative layer chromatography on silica gel (E. Merck, 0.25 mm). They are described in order of increasing polarity.

Eremantholide C (6) was obtained with properties as previously described.[11]

Compound 9. This was obtained as an oil: UV (EtOH) λ_{max} 266 nm (log ϵ 3.67); IR (film) 1770 (γ-lactone), 1705 (enone C=O), 1650, 1585 cm^{-1} (C=C); ^1H NMR ($CDCl_3$) δ 6.00–5.80 (2 H, m), 5.60 (1 H, s), 4.86 (1 H, m), 4.00–3.66 (1 H, m), 3.40–3.06 (2 H, dq), 2.80 (1 H, dd, J = 8, 4 Hz), 2.03 (3 H, s), 1.66–1.56 (6 H, d), 1.46 (3 H, s), 1.26–0.92 (6 H, m); mass spectrum, m/e 388.1924 (M^+; calcd for $C_{22}H_{28}O_6$, 388.187).

Compound 8. This compound was present along with compound 9 in several fractions from the column and was further purified by preparative TLC (E. Merck, 0.25 mm, 3:1 hexane–ethyl acetate). Compound 8 is an oil: UV (EtOH) λ_{max} 265 nm (log ϵ 3.96); IR (film) 1770 (γ-lactone), 1705 (enone C=O), 1650, 1585 (C=C); ^1H NMR ($CDCl_3$) δ 6.00–5.83 (2 H, m), 5.60 (1 H, s), 4.80 (1 H, m), 3.67 (1 H, m), 2.90 (3 H, s), 2.02 (3 H, s), 1.65 (6 H, br s), 1.48 (3 H, s), 1.09 (3 H, s); mass spectrum, m/e 374.174 (M^+; calcd for $C_{21}H_{26}O_6$, 374.172).

Compound 7. Further purification of fractions close to those containing eremantholide C (6) with preparative TLC plates (E. Merck, 0.25 mm) gave compound 7: mp 220–222 °C (EtOH–acetone); $[\alpha]^{24}_D$ -31° (c 0.6, EtOH); UV (EtOH) λ_{max} 266 nm (log ϵ 4.01); IR (film) 3450 (OH), 1770 (γ-lactone), 1695 (enone C=O), 1650, 1580 cm^{-1} (C=C); ^1H NMR ($CDCl_3$) δ 6.03–5.80 (2 H, s), 5.60 (2 H, s), 5.03 (1 H, m), 4.03 (1 H, m), 3.66 (1 H, br, s), 1.73–1.63 (6 H, d), 1.40 (3 H, s), 1.10 (3 H, s); mass spectrum, m/e 360.158 (M^+; calcd for $C_{20}H_{24}O_6$, 360.156).

Lychnophorolide A (3). After recrystallization from ethanol–ethyl acetate this compound had the following: mp 165.5–166.5 °C; $[\alpha]^{24}_D$ -34° (c 1.4, EtOH); UV (EtOH) λ_{max} 266 nm (log ϵ 4.20), 214 (4.46); IR (KBr) 3400 (OH), 1765, 1650 (α,β-unsaturated γ-lactone), 1695 (enone C=O), 1580 (enone C=C), 1140 cm^{-1} (COC); ^1H NMR ($CDCl_3$) δ 6.20–6.00 (3 H, m), 5.80 (1 H, s), 5.46 (1 H, d, J = 2 Hz), 5.36 (1 H, m), 4.56 (1 H, m), 4.40 (2 H, br s), 3.80 (1 H, m), 3.30 (1 H, br, s, OH, exchanged with D_2O), 1.93–1.80 (6 H, d), 1.53 (3 H, s); mass spectrum, m/e 374.134 (M^+; calcd for $C_{20}H_{22}O_7$, 374.135).

Lychnophorolide B (4). Purification of this compound by preparative TLC (E. Merck, 0.25 mm, 3:1 hexane–ethyl acetate) gave an homogeneous oil (4 mg) which resisted attempted crystallization: UV (EtOH) λ_{max} 266 nm (log ϵ 3.87), 212 (4.15); IR (KBr) 3400 (OH), 1765, 1650 (α,β-unsaturated γ-lactone), 1695 (enone C=C), 1580 (enone C=C), 1140 cm^{-1} (COC); ^1H NMR ($CDCl_3$) δ 6.71 (1 H, q), 6.21 (1 H, m), 5.80 (1 H, s), 6.13 (1 H, d, J = 2 Hz), 5.73 (1 H, s), 5.38 (1 H, d, J = 2 Hz), 5.28 (1 H, m), 4.33 (2 H, br s), 3.72 (1 H, m), 1.70–1.65 (6 H, d), 1.46 (3 H, s); mass spectrum, m/e 374.134 (M^+; calcd for $C_{20}H_{22}O_7$, 374.135).

Compound 11. A few of the more polar fractions eluted from the column, after further chromatographic purification on silica gel 60, gave materials containing the 3(2*H*)-furanone system (IR ν_{max} 1580 cm^{-1}). These fractions were again chromatographed on a column (silica gel 60) to give compound 11: IR (film) 3400 (OH), 1770 (γ-lactone), 1705 (enone C=O), 1660, 1590 (C=C); ^1H NMR ($CDCl_3$) δ 6.26 (1 H, m), 5.83 (1 H, m), 5.70 (1 H, s), 4.93 (1 H, m), 4.30 (2 H, br s), 3.90 (1 H, m), 3.46–3.06 (2 H, dq), 2.90 (1 H, dd, J = 8, 4 Hz), 1.66–1.56 (6 H, d), 1.46 (3 H, s), 1.26–0.93 (6 H, m); mass spectrum, m/e 404.184 (M^+; calcd for $C_{22}H_{28}O_7$, 404.182).

X-ray Analyses of Lychnophorolide A (3). Lychnophorolide A ($C_{20}H_{22}O_7$, mol wt 374.39) crystallized, by slow evaporation from aqueous ethanol, in the monoclinic space group $P2_1$ with unit cell constants a = 9.343 (2) Å, b = 11.808 (4) Å, c = 8.500 (2) Å, β = 95.57 (2)° and d_{calcd} = 1.332 g cm^{-3} (Z = 2). Integrated intensities for 1070 unique reflections with $2\theta \leq 45$ were measured on a Syntex $P2_1$ automated diffractometer by using graphite-monochromated MoKα radiation (λ = 0.71069 Å) and a variable-speed $\theta/2\theta$ scan technique. Of these, 948 were considered observed with $I \geq 1.5\sigma(I)$. Lorentz and polarization factors were applied; absorption corrections were deemed unnecessary (μ = 8.54 cm^{-1}).

Initial attemps to determine the structure with the MULTAN[18] program package were unsuccessful. The structure was solved by using the program QTIN[19] which generated more than 8000 phase ambiguities. A recognizable molecular fragment was found in the E map corresponding to the solution with the lowest NQUEST[20] figure of merit. The remaining nonhydrogen atoms were located in subsequent difference Fourier maps. Nonhydrogen atoms were refined with anisotropic thermal parameters by full-matrix least-squares cycles. Hydrogen atoms in calculated positions with an isotropic B of 4.0 Å^2 were included in the structure factor calculation but were not refined. A difference Fourier synthesis revealed only half of the hydrogen atoms. Convergence was reached at an R of 0.055 for the observed data.

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Registry No. 1, 68945-57-3; 2, 77836-74-9; 3, 80795-27-3; 4, 80795-28-4; 6, 69883-97-2; 7, 79389-83-6; 8, 80754-68-3; 9, 80754-69-4; 11, 80754-70-7.

Supplementary Material Available: Tables I and II, listing final atomic positional and anisotropic thermal parameters for the nonhydrogen atoms, Table III, listing calculated positional parameters for hydrogen atoms, and Tables IV and V, listing bond lengths and angles and torsion angles (6 pages). Ordering information is given on any current masthead page.

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