Antitumour Plants. Part 6.[1,2] Novel Modified Germacranolides and Other Constituents of *Eremanthus elaeagnus* Schultz-Bip (Compositae)

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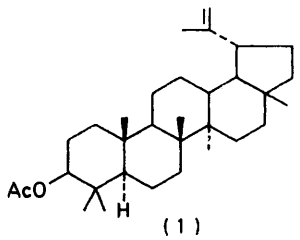
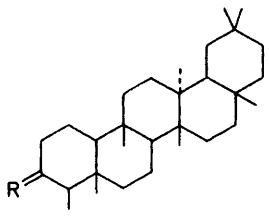
Extracts of the stem parts of the woody Composite *Eremanthus elaeganus* yielded lupeol acetate (1), friedelin (2), epifriedelinol (3), a mixture of sitosterols, the flavonoids tamarixetin (3,5,7,3'-tetrahydroxy-4'-methoxyflavone), genkwanin (5,4'-dihydroxy-7-methoxyflavone), and luteolin 7-methyl ether (5,3',4'-trihydroxy-7-methoxyflavone), and the three novel modified germacranolides eremantholide A (5), B (6), and C (minor component) (7). The structures of eremantholides A and B were determined by *X*-ray crystallography, and a crystallographic comparison between the two structures is given. Eremantholide A reacts with propane-1-thiol by a novel 1,6-Michael-type addition which may simulate the mode of its cytotoxic action.

THE family *Compositae* has long been known as a rich source of diverse natural products, such as polyacetylenes, alkaloids, flavonoids, and sesqui-, di-, and triterpenoids.[3] In recent years considerable attention has been paid to the sesquiterpenoid lactones characteristic of most tribes of the family because of the cytotoxicity, and potential antitumor activity[4] which many of them display. In this paper we report our investigation of the stem parts of *Eremanthus elaeagnus* (Schultz-Bip.) (Vernoniae), one of the rare woody composites, which has led to the isolation of several triterpenoids, three novel modified germacranolides, and some known flavonoids.

The dried plant was extracted and partitioned according to established procedures[5] into fractions soluble in light petroleum, methanol–water (90 : 10), and water–methanol (90 : 10). The light petroleum fraction was further divided into neutral and acidic portions. Chromatography of the neutral portion on alumina gave, in order of appearance on gradient elution, lupeol acetate (1), friedelin (2), and epifriedelinol (3). Following these



(1)



(2) R = O
(3) R = β-OH, α-H

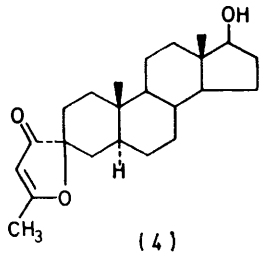
compounds was a substance which appeared to be a mixture. It resisted further attempted separations, but on the basis of the following evidence it is assumed to be a mixture of an amyrin and possible a lupeol derivative.

[1] Part 5, R. F. Raffauf, P. C. Ghosh, and P. W. Le Quesne, *Lloydia* (*J. Nat. Prods.*), in the press.
[2] Preliminary communication, R. F. Raffauf, P.-K. C. Huang, P. W. Le Quesne, S. B. Levery, and T. F. Brennan, *J. Amer. Chem. Soc.*, 1975, **97**, 6884.
[3] R. Hegnauer, 'Chemotaxonomie der Pflanzen,' Birkhäuser Verlag, Basel, 1964, vol. 3, p. 443.
[4] For a review, see E. Rodriguez, G. H. N. Towers, and J. C. Mitchell, *Phytochemistry*, 1976, **15**, 1573.
[5] For a typical extraction sequence see P. C. Ghosh, J. E. Larrahondo, P. W. Le Quesne, and R. F. Raffauf, *Lloydia* (*J. Nat. Prods.*), 1977, **40**, 364.

The mass spectrum showed molecular weight 426.387 ($C_{30}H_{50}O$ requires 426.386), suggesting the likelihood of a pentacyclic triterpenoid with one double bond. A pink colour with Liebermann–Burchard reagent supported this idea, but the n.m.r. spectrum showed distinctly different, although poorly defined, olefinic absorptions at δ 5.2 and 4.6. The possibility of a tetracyclic structure with another double bond was ruled out by detailed examination of the high-resolution mass spectrum, which was in good accord with the data given for amyrin derivatives by Djerassi and his co-workers.[6] Major peaks observed at *m/e* 218.204 (100%; $C_{16}H_{26}$ requires 218.203) and 207.176 ($C_{14}H_{23}O$ requires 207.175) are ascribed to the characteristic retro-Diels–Alder cleavage of ring C in the Δ^{12}-oleanene series. Further fragmentations of these ions, and other ions in the spectrum, were fully consistent with this structural type. It is likely, therefore, that this substance is an amyrin mixed with an isomeric pentacyclic triterpene alcohol, perhaps lupeol, in view of the isolation of lupeol acetate above. A mixture of sitosterols was eluted finally from the column.

The methanol–water (90 : 10) fraction in initial experiments [2] was chromatographed directly on silica gel, and after extensive chromatography followed at all stages by bioassay *vs.* 9 KB,[7] eremantholides A and B were obtained. In later experiments, we found that isolation of the eremantholides could be greatly facilitated by first removing acidic and polyphenolic substances from the fraction and then seeding an ethanolic solution of the residue with crystalline eremantholide A. The crystals obtained in this way were a mixture of two compounds, eremantholide A (5) ($C_{19}H_{24}O_6$; 81%), and eremantholide B (6), ($C_{20}H_{26}O_6$; 19%). These compounds were obtained pure by high-pressure liquid chromatography. Each compound showed activity *vs.* 9 KB at 2 μg/ml.

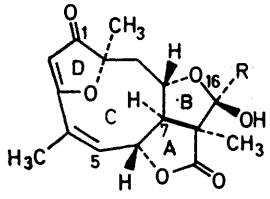
The u.v. spectrum of eremantholide A showed $\lambda_{max.}$ EtOH 266 nm (log ε 4.00), which is characteristic of furenones such as (4).[8] The i.r. spectrum showed major absorptions at 3 400 (O–H), 1 770 (γ-lactone), 1 695 (enone C=O), 1 653, and 1 582 cm^{-1} (C=C). Eremantholide A is neutral, and was unaffected by acetic anhydride–pyridine, although trimethylsilyl chloride–pyridine converted it into a mono-trimethylsilyl derivative. The base peak in the mass spectrum of eremantholide A arises from monodehydration (*m/e* 330.146; $C_{19}H_{22}O_5$ requires 330.146). These data suggested a tertiary alcohol, a saturated γ-lactone, and an enone function. The remaining two oxygen atoms were assigned to ether functions when micro-hydrolysis experiments, by giving no small fragments, ruled out an ester side-chain. The n.m.r. spectrum of eremantholide A shows four protons resonating below δ 4.0, *i.e.* a 1 H multiplet at δ 6.0, a sharp 1 H singlet at δ 5.60, a 1 H multiplet at δ 4.9, and a six-line 1 H signal at δ 4.0. The O–H signal occurs as a sharp singlet (confirmed by D_2O exchange) at δ 2.75, overlying a 1 H doublet of doublets centred at δ 2.8. An allylic methyl signal, somewhat split by long-range coupling, falls at δ 2.0, as well as two methyl singlets at δ 1.45 and 1.35. Centred at δ 1.0 are two superimposed 3 H doublets



(4)

from an isolated isopropyl group. This group is readily lost on electron impact, giving rise to a large peak at *m/e* 305.104 ($C_{16}H_{17}O_6$ requires 305.097). These data suggest the presence of a $Me_2CHC(O)$– function undergoing ready mass spectrometric α-cleavage.

The spectral characteristics of eremantholide B were almost identical with those of eremantholide A; in particular, the same peak at *m/e* 305 was present, in both mass spectra, and the two spectra at lower *m/e* values were identical. The n.m.r. spectrum of eremantholide B was slightly different in the δ 1 region, suggesting that this compound has a s-butyl or isobutyl substituent instead of the isopropyl group. *X*-Ray structure determinations of both compounds were performed



(5) R = –⟨

(6) R = –⟨

(7) R = –⟨

and revealed the structures of eremantholides A and B to be (5) and (6) respectively.

Crystals of the sesquiterpenoids were obtained by slow evaporation of ethanolic solutions. Crystal data are given in Table 1. Three dimensional intensity data

TABLE 1

Crystal data for eremantholides A and B

	Eremantholide A	Eremantholide B
Mol. formula	$C_{19}H_{24}O_6$ (348.16)	$C_{20}H_{26}O_6$ (362.18)
Space group	$P2_1$	$P2_12_12_1$
Cell dimensions	a 10.242(2) Å	a = 8.939(2) Å
	b = 10.397(3)	b = 10.281(4)
	c = 8.965(2)	c = 20.600(7)
	β = 98.08(2)°	
	V = 945.2 Å^3	V = 1 893.2 Å^3
	Z = 2	Z = 4
Calculated density	ρ = 1.22 g cm^{-3}	ρ = 1.27 g cm^{-3}
Range of data	$2\theta_{max}$ = 48° (Mo-Kα)	$2\theta_{max}$ = 45°
Observed (total) reflections	1 035 (1 338)	1 058 (1 457)

[6] H. Budzikiewicz, J. M. Wilson, and C. Djerassi, *J. Amer. Chem. Soc.*, 1963, **85**, 3688.

[7] R. I. Geran, N. H. Greenberg, M. M. Macdonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemotherapy Reports*, 1972, **3**, 1.

[8] P. K. Gupta, J. G. Ll. Jones, and E. Caspi, *J. Org. Chem.*, 1975, **40**, 1420.

were collected by the θ—2θ scan method on a Syntex P_{2_1} automated diffractometer using monochromatized Mo-K_α radiation (λ = 0.710 69 Å). Lorentz and polarization corrections were applied to both sets of data.

TABLE 2

Atomic parameters of eremantholide A

Atom	X	Y	Z
O(1)	0.575 7(8) [a]	0.712 8	0.539 8(8)
O(2)	0.743 7(7)	0.588 7(7)	0.501 3(7)
O(3)	1.025 7(6)	0.118 0(8)	0.910 2(7)
O(4)	0.798 6(5)	0.147 9(6)	0.579 3(6)
O(5)	0.692 6(5)	0.414 9(6)	0.901 4(6)
O(6)	0.778 1(5)	0.611 2(6)	0.840 0(6)
C(1)	0.968 4(8)	0.143 8(10)	0.786 0(11)
C(2)	1.011 2(8)	0.205 9(11)	0.659 1(11)
C(3)	0.907 1(9)	0.210 8(10)	0.548 2(14)
C(4)	0.884 4(9)	0.282 5(12)	0.405 3(14)
C(5)	0.833 8(10)	0.398 0(11)	0.410 2(10)
C(6)	0.802 4(9)	0.463 6(9)	0.550 0(9)
C(7)	0.698 7(7)	0.400 3(9)	0.638 0(8)
C(8)	0.759 9(7)	0.343 7(9)	0.795 8(9)
C(9)	0.740 5(8)	0.201 0(9)	0.820 6(10)
C(10)	0.822 9(8)	0.112 4(9)	0.739 5(9)
C(11)	0.609 2(7)	0.510 1(8)	0.674 2(9)
C(12)	0.635 9(9)	0.616 3(9)	0.569 3(9)
C(13)	0.464 0(8)	0.476 6(10)	0.648 2(11)
C(14)	0.791 9(11)	−0.029 5(11)	0.758 6(12)
C(15)	0.916 8(12)	0.218 5(12)	0.263 8(11)
C(16)	0.660 8(7)	0.537 5(9)	0.842 9(9)
C(17)	0.569 0(7)	0.601 5(9)	0.941 6(10)
C(18)	0.529 1(10)	0.734 6(10)	0.892 4(12)
C(19)	0.628 2(12)	0.594 3(15)	1.109 4(12)
H(06)	0.835	0.625	0.940
H(2)	1.093	0.238	0.658
H(5)	0.816	0.447	0.333
H(6)	0.901	0.486	0.626
H(7)	0.641	0.336	0.576
H(8)	0.834	0.357	0.819
H(141)	0.871	0.274	0.151
H(142)	0.865	0.139	0.252
H(143)	0.995	0.193	0.243
H(17)	0.496	0.555	0.927
H(91)	0.657	0.179	0.794
H(92)	0.749	0.182	0.931
H(131)	0.449	0.401	0.707
H(132)	0.433	0.456	0.551
H(133)	0.403	0.547	0.672
H(151)	0.690	−0.051	0.728
H(152)	0.815	−0.044	0.861
H(153)	0.842	−0.090	0.689
H(181)	0.491	0.740	0.792
H(182)	0.600	0.795	0.902
H(183)	0.458	0.769	0.960
H(191)	0.705	0.656	1.118
H(192)	0.672	0.488	1.155
H(193)	0.576	0.630	1.167

[a] Estimated standard deviations in parentheses.

Both acentric structures were solved by direct methods through application of the MULTAN program package, which employs a multiple solution-tangent refinement procedure. The eremantholide A structure was solved by MULTAN using 235 reflections with $|E|$ values greater than 1.40. The 25 non-hydrogen atoms of the structure were located among the 29 highest peaks of the E map generated by the solution having the highest figure of merit. The structure determination of eremantholide B was less straight-forward. Initial difficulty in obtaining a reasonable solution with MULTAN was overcome only after using the structure results of the closely related eremantholide A to calculate $|E|$ values by the Debye curve method. Using 216 reflections with $|E|$ values greater than 1.35, the 26 non-hydrogen atoms of the molecule were found among the 29 highest peaks of the E map corresponding to the 'best' solution. Following full-matrix least squares refinement of the non-hydrogen atoms with anisotropic temperature factors, all hydrogen atoms for both structures were located in difference Fourier maps. Further refinement resulted in final weighted and unweighted R factors, respectively, of 0.056 and 0.057 for eremantholide A (5) and 0.061 and 0.067 for eremantholide B (6). The final positional parameters of the non-hydrogen atoms and hydrogen atoms are given in Tables 2 and 3. Atomic scattering factors for the C and O atoms were taken from the International Tables for X-ray Crystallography (1974) and those for H atoms from Stewart, Davidson,

TABLE 3

Atomic parameters of eramantholide B

Atom	X	Y	Z
O(1)	0.069 5(6) [a]	0.543 1(6)	0.508 6(3)
O(2)	0.025 8(6)	0.458 4(6)	0.411 4(3)
O(3)	0.484 6(8)	0.103 8(7)	0.219 5(3)
O(4)	0.193 0(6)	0.067 5(6)	0.332 5(3)
O(5)	0.472 9(5)	0.359 9(5)	0.404 7(2)
O(6)	0.340 6(6)	0.554 0(5)	0.389 1(2)
C(1)	0.365 6(11)	0.103 0(10)	0.249 7(4)
C(2)	0.218 9(10)	0.155 9(12)	0.231 4(4)
C(3)	0.131 5(10)	0.135 9(9)	0.282 9(4)
C)4)	−0.020 2(10)	0.188 3(9)	0.297 2(4)
C(5)	−0.032 0(10)	0.288 9(10)	0.336 5(4)
C(6)	0.093 2(10)	0.368 2(9)	0.366 7(4)
C(7)	0.217 0(8)	0.297 0(7)	0.405 2(4)
C(8)	0.368 6(9)	0.282 7(8)	0.369 8(4)
C(9)	0.432 3(9)	0.144 7(8)	0.366 3(4)
C(10)	0.351 7(9)	0.054 5(8)	0.318 8(4)
C(11)	0.252 0(8)	0.392 4(7)	0.461 0(4)
C(12)	0.112 4(9)	0.475 3(7)	0.464 0(4)
C(13)	0.276 5(9)	0.323 5(7)	0.525 2(4)
C(14)	0.397 2(12)	−0.087 3(9)	0.326 6(5)
C(15)	−0.148 4(10)	0.121 6(10)	0.263 4(5)
C(16)	0.394 0(8)	0.463 0(8)	0.435 9(4)
C(17)	0.5 000(9)	0.526 6(8)	0.484 0(4)
C(18)	0.645 0(10)	0.570 6(10)	0.450 7(5)
C(19)	0.427 9(10)	0.642 0(9)	0.519 5(4)
C(20)	0.510 4(14)	0.685 7(9)	0.579 3(4)
H(06)	0.398	0.576	0.370
H(2)	0.198	0.210	0.189
H(5)	−0.118	0.323	0.346
H(6)	0.119	0.425	0.335
H(7)	0.180	0.205	0.420
H(8)	0.359	0.322	0.326
H(91)	0.420	0.097	0.411
H(92)	0.547	0.138	0.352
H(131)	0.357	0.266	0.522
H(132)	0.184	0.268	0.536
H(133)	0.303	0.388	0.563
H(141)	0.372	−0.107	0.375
H(142)	0.497	−0.095	0.319
H(143)	0.333	−0.136	0.292
H(151)	−0.249	0.152	0.274
H(152)	−0.144	0.032	0.283
H(153)	−0.152	0.113	0.205
H(17)	0.525	0.450	0.516
H(181)	0.613	0.646	0.420
H(182)	0.667	0.513	0.433
H(183)	0.724	0.599	0.484
H(191)	0.326	0.610	0.529
H(192)	0.422	0.716	0.493
H(201)	0.636	0.713	0.563
H(202)	0.526	0.621	0.616
H(203)	0.487	0.762	0.601

[a] Estimated standard deviations in parentheses.

and Simpson.[9] The least-squares refinements were carried out using Stewart's XRAY76 crystallographic program system. The structure factors and thermal parameters for both compounds are deposited as a Supplementary Publication (No. SUP 22339, 16 pp.)*

The molecular conformation [10] of each structure is given in Figure 1. Bond distances, bond angles, and conformational angles for both compounds are given in Figures 2 and 3. The overall molecular conformations are quite similar although there are some significant differences. In both molecules the fused A and B rings are puckered and inclined towards one another at *ca.* 127°; however, the mode of puckering differs. In (5), the A ring displays an envelope conformation with C-7 displaced from the least-squares sphere of the remaining four atoms of the ring by 0.31 Å. The B ring exhibits a twist conformation with atoms C-16 and O-5 displaced 0.56 and 0.48 Å, respectively, on opposite sides of the plane formed by C-7, C-8, and C-11. In (6), the A ring has a twist conformation with displacements of 0.37 and 0.31 Å for C-7 and C-6 respectively, and the B-ring exhibits an envelope conformation with a C-16 displacement of 0.47 Å. These differences in ring puckering are reflected in the A and B ring torsion angles (see Figure 3), which differ by an average of *ca.* 4° for the two molecules. The D-rings of both molecules are essentially planar. The conformational differences between (5) and (6) are largest in the C-ring, primarily about the C(3)–C(4) and C(7)–C(8) bonds. The constraints imposed by the four-ring fusion apparently require that the double bond be markedly out of plane; the corresponding torsion angle is 88.6° in (5) A and 99.4° in (6). The differences in geometry of rings A and B between the two compounds lead to significant differences in the C(6),C(7),C(8),C(9) torsion angles—they are 127.4 and 120.8° for (5) and (6) respectively. As a result of these differences, the orientation of the furanone D ring with respect to the rest of the molecule differs somewhat in the two eremantholides (see Figure 1). All bond distances and angles fall within the range of normally expected values.

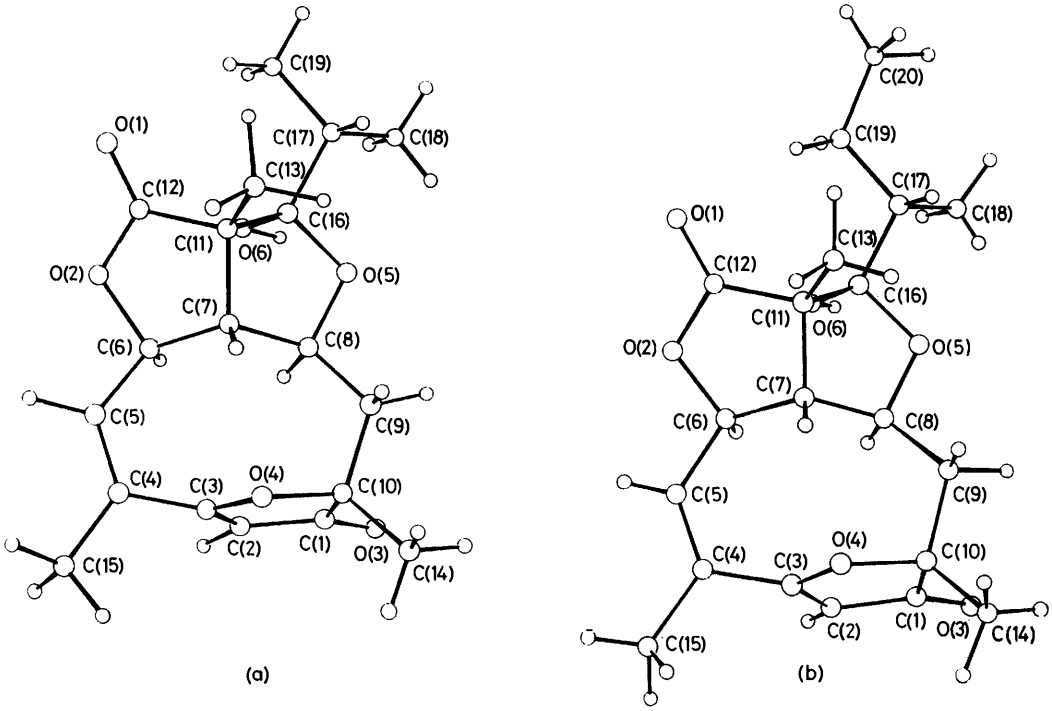


FIGURE 1 ORTEP drawings showing the general molecular conformation of (a) eremantholide A and (b) eremantholide B

Eremantholide A and B crystallize in different space groups, P_{2_1} and $P_{2_12_12_1}$ respectively, and thus exhibit different intermolecular packing schemes. However, in both crystals, hydrogen bonds between O(3) · · · H–O(6) atoms of two-fold screw axis related molecules along the *b* axis are the dominant interactions between molecules (see Figure 4). No other intermolecular interactions are present and there are no intermolecular distances among non-hydrogen atoms less than 3.30 Å.

The similarity in overall conformations of these two molecules as determined in the crystalline state reflects the constraints imposed upon the cyclodecadienone C-ring by the A,B-ring fusion and by the D-ring, incorporating the oxygen bridge. The small conformational differences in the A, B, and C rings indicate the narrow range of flexibility of the ring system, and appear to arise

* See Notice to Authors No. 7, *J.C.S. Perkin I*, 1977, Index issue for details of the Scheme.

[9] R. F. Stewart, E. R. Davidson, and W. T. Simpson, *J. Chem. Phys.*, 1965, **42**, 3175.

[10] C. K. Johnson, ' ORTEP,' Report ORNL–3794, Oak Ridge National Laboratory, Oak Ridge, Tennessee, 1965.

from the different packing arrangements. It would, therefore, be expected that solution conformations of the eremantholides should depart little from those in the solid state; this is corroborated by the fact that the u.v. spectra show no influence of conjugation between the furanone chromophore and the adjacent double bond. Moreover, from the n.m.r. spectra of (5) and (6) in chloroform solution, there is no tendency for these molecules to exist in solution as ring-opened ketol tautomers of the hemiacetals. The hemiacetal functions clearly facilitate the mass spectral α-cleavage of the isopropyl and s-butyl groups.

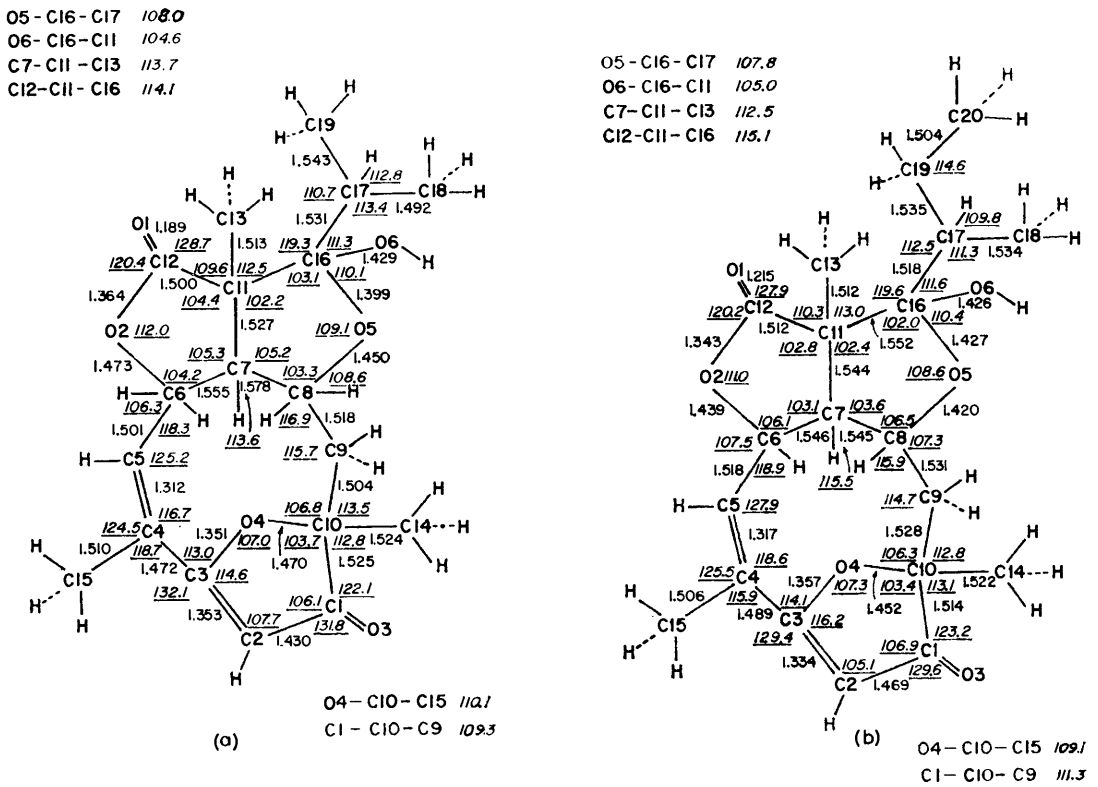


FIGURE 2 Interatomic bond distances and angles in (a) eremantholide A (average standard deviations are 0.010 Å and 0.6°) and (b) eremantholide B (average standard deviations are 0.009 Å and 0.6°)

In the n.m.r. spectrum of (5), the multiplet at δ 6.0 is assigned to 5-H, and the sharp singlet at δ 5.60 to 2-H; the analogous proton in (4) resonates at δ 5.21, but in eremantholide A this proton may be further deshielded by the 4,5-double bond. The multiplets at δ 4.9 and 4.0 are assigned to 6-H and 8-H respectively, while 7-H gives rise to the doublet of doublets signal (J = 8 Hz, 4 Hz) at δ 2.8. The C-15 allylic methyl group gives rise to the broadened 3H-signal at δ 2.0, while the upfield 3 H singlets at δ 1.45 and 1.35 are assigned to the C-14 and C-13 methyl groups respectively. The spectral data are almost coincident in (6) and the interpretation is the same.

Chromatography of the mother liquors remaining after crystallization of the eremantholide A/B mixture yielded, as well as more of these compounds, eremantholide C, $C_{19}H_{22}O_6$. This compound, which is laevorotatory, in contrast to the eremantholides A and B, has u.v. and n.m.r. spectra very similar to those of (5) and (6); the n.m.r. spectrum clearly shows an isopropenyl rather than an isopropyl side-chain. A 2H-multiplet at δ 5.18 arises from a terminal methylene group in an asymmetric environment, and a new allylic methyl singlet appears at δ 2.18. In the mass spectrum of

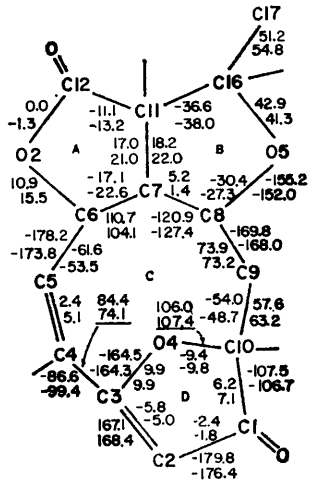


FIGURE 3 Torsion angles involving the atoms of the four fused rings of eremantholide A (upper values) and eremantholide B (lower values). Endocyclic conformational angles are written inside and exocyclic angles outside the rings

eremantholide C, major peaks arise from the molecular ion (346.142; $C_{19}H_{22}O_6$ requires 346.142) by dehydration (328.134; $C_{19}H_{20}O_5$ requires 328.131), loss of CO_2 (302.152; $C_{18}H_{22}O_4$ requires 302.152), and loss of C_3H_6 (isopropenyl + H) from this latter ion (260.106; $C_{15}H_{16}O_4$ requires 260.105). As expected, the peak at *m/e* 305 found in (5) and (6) was very weak in the spectrum of eremantholide C. From these data, eremantholide C is assigned structure (7), the configuration at C-16 being assumed to be the same as in (5) and (6).

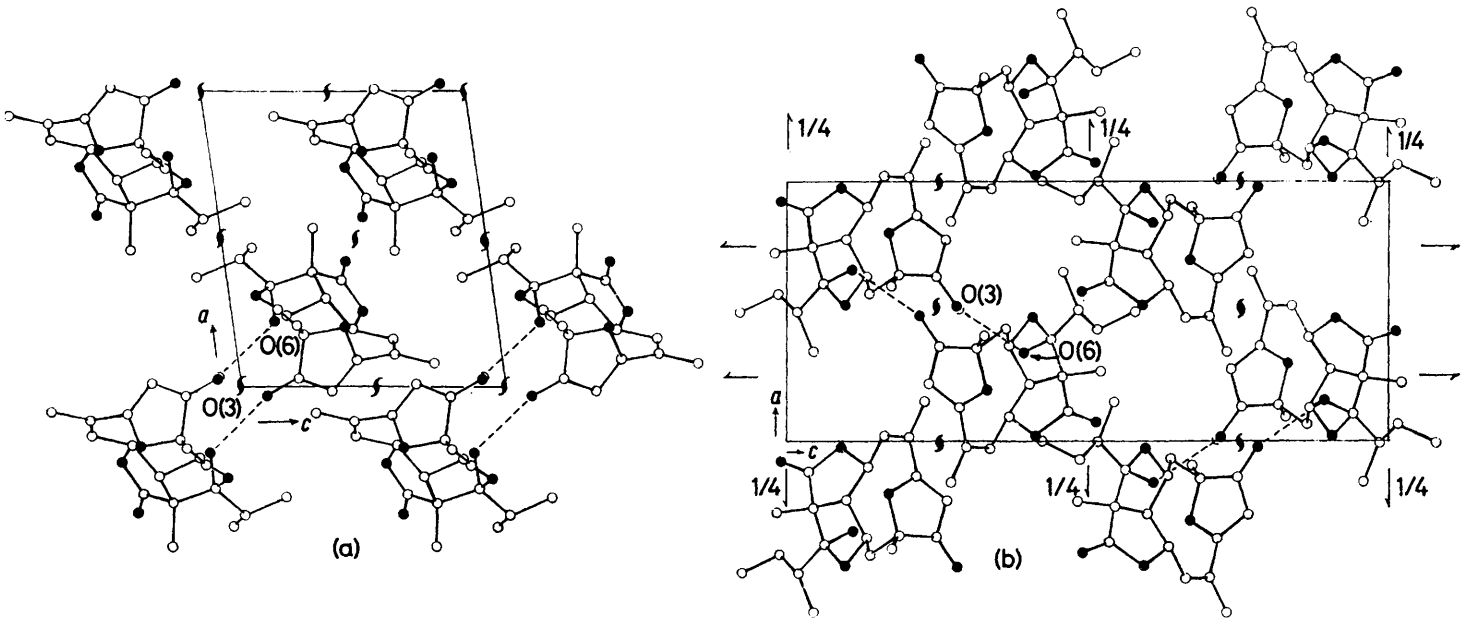
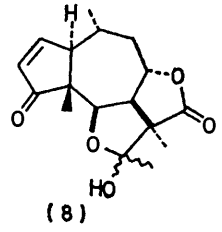


FIGURE 4 The molecular packing of (a) eremantholide A and (b) eremantholide B viewed down the *b* screw axis. Intermolecular hydrogen bonds are denoted by the broken lines



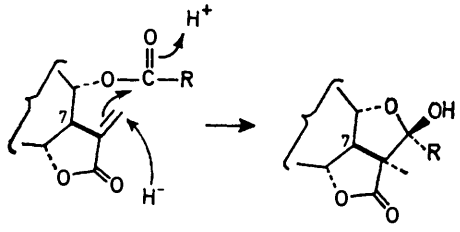
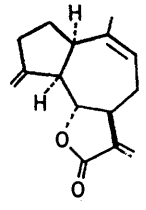
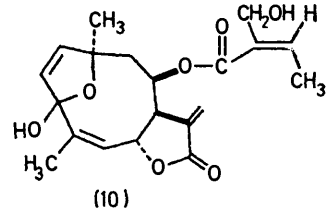
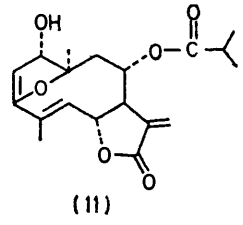


FIGURE 5 Proposed biogenesis of the eremantholides

The structures of eremantholides B and C, (6) and (7), lend support to our view, reported earlier,[3] of the biogenesis of (5) and of tenulin (8).[11] This involves a reductive intramolecular addition (see Figure 5) uniting an α-methylene-γ-lactone function and an adjacent α-acyloxy-side chain. Easily achieved variations in the side-chain would lead to the different eremantholides; and a 6-acetoxy-side chain would be envisaged for the tenulin precursor. The heartwood of *E. elaeagnus* contains eremanthine (9),[12] a schistosomicidal sesquiterpene lactone, and compounds related to the eremantholides of obviously sesquiterpenoid structure have







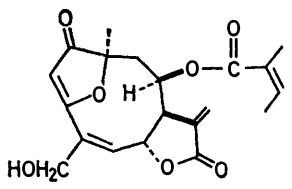
recently been reported. These observations strongly support the idea [2] that the eremantholides are novel transformed germacranolide derivatives, rather than

[11] W. Herz and R. P. Sharma, *J. Org. Chem.*, 1975, **40**, 2557, and references cited therein.

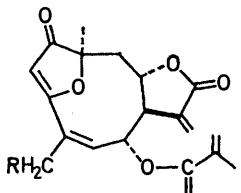
[12] P. M. Baker, C. C. Fortes, E. G. Fortes, G. Gazinelli, B. Gilbert, J. N. C. Lopes, J. Pellegrino, T. C. B. Tomassini, and W. Vichnewski, *J. Pharm. Pharmacol.*, 1972, **24**, 853; W. Vichnewski and B. Gilbert, *Phytochemistry*, 1972, **11**, 2563; M. Garcia, A. J. R. Silva, P. M. Baker, B. Gilbert, and J. A. Rabi, *ibid*, 1976, **15**, 331.

modified diterpenoids. Particularly noteworthy relatives of the eremantholides are liatrin (10),[13] ciliarin (11),[14] budlein-A (12),[15] goyazenosolide (13),[16] and 15-deoxygoyazensolide (14).[17]

The cytotoxicity of the eremantholides is particularly

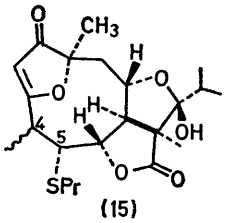


(12)



(13) R = OH
(14) R = H

interesting in view of the *absence* of the αβ-unsaturated γ-lactone function which has consistently been implicated[4] as the site of nucleophilic attack by enzymes and/or nucleic acids. We have treated eremantholide A with propane-1-thiol in tetrahydrofuran/pH 9.2 borate buffer and obtained a crystalline 1 : 1 adduct, m.p. 236—241 °C. The i.r. spectrum of this product showed major absorptions at 1 770, 1 700, and 1 585 cm^{-1}, suggesting that both γ-lactone and furanone functions are intact. The 1 653 cm^{-1} absorption of eremantholide A was lacking. The mass spectrum (M^+ 424.182; $C_{22}H_{32}O_6S$ requires 424.192) showed peaks for dehydration (406.180; $C_{22}H_{30}O_5S$ requires 406.181), loss of *S*-propyl (349.164; $C_{19}H_{25}O_6$ requires 349.165), and propanethiol (low intensity; 348.157; $C_{19}H_{24}O_6$ requires 348.157), and loss of water and the *S*-propyl group (331.153; $C_{19}H_{23}O_5$ requires 331.154). In the n.m.r. spectrum the 5-H signal was absent and 2-H was shifted upfield to δ 5.45. A 2 H multiplet at δ 4.4 is assigned to 6-H and another hydrogen. The C-13 and C-14 methyl groups appear at δ 1.4 and four other methyl groups appear near δ 1.0. These data are consonant with structure (15) for the adduct; we suggest that the com-



(15)

pound arises by a novel 1,6-Michael-type addition from the α-face of the molecule. The stereochemistry of the methyl group at C-4 is not certain, and our material may not be homogeneous in this respect. The formation of the adduct by a Michael-type process would involve some twisting of the furanone ring in order to confer polarization on the 4,5-double bond in eremantholide A; although the reaction between the sesquiterpenoid and propane-thiol was slow (several days in solution at 20 °C) we favour a Michael-type process rather than a free radical-mediated addition to the 4,5-double bond. Further work is in progress.

Chromatography of the sodium hydrogen carbonate-soluble material from the methanol–water (90 : 10) fraction gave a flavonol whose properties are in accord with its being tamarixetin (3,5,7,3′-tetrahydroxy-4′-methoxyflavone). The sodium carbonate-soluble material yielded two flavonoids, genkwanin (5,4′-dihydroxy-7-methoxyflavone) and luteolin-7-methyl ether (5,3′,4′-trihydroxy-7-methoxyflavone). The fraction of the plant extractives soluble in methanol–water (10 : 90) was inactive *vs.* KB and was not investigated in detail.

EXPERIMENTAL

General experimental details are given in ref. 5.

Extractions and Partitions.—The dried, ground stem (2.5 kg) was extracted exhaustively with 95% ethanol at room temperature. The ethanolic extract was concentrated under reduced pressure below 45 °C to a thick syrup, which was partitioned between chloroform (5 l) and water–methanol (9 : 1) (5 l). The aqueous layer was concentrated under reduced pressure to remove methanol and some water, and then freeze-dried (wt. 38.2 g). The chloroform layer was concentrated under reduced pressure to a thick syrup and partitioned between light petroleum (4 l) and methanol–water (9 : 1) (4 l). The methanolic fraction was then concentrated under reduced pressure and finally freeze-dried (wt. 91.6 g). The light petroleum extract was concentrated to a thick syrup and air-dried (wt. 62.9 g). Additional quantities of these fractions [light petroleum, 97 g; methanol–water (9:1), 198.6 g; water–methanol (9 : 1), 15.4 g] became available from other extraction sequences and were combined with the materials above.

Constituents of the Light Petroleum Fraction.—The crude fraction, by partitioning between 5% aqueous sodium hydrogen carbonate solution and ether, gave acidic (6%) and neutral (91%) material.

Chromatography of the neutral material. The neutral material (40 g) was chromatographed on neutral alumina (Woelm, activity III, 3 kg) in a 7 ft × 2.5 in column. Gradient elution (hexane–benzene–chloroform–acetone–methanol as developing solvents) was employed, and 68 fractions were collected. Each fraction (2 l) was concentrated to dryness and examined by t.l.c. on silica gel. Fractions were combined on the basis of their t.l.c. behaviour.

Fractions 1 and 2 (hexane) yielded saturated hydrocarbons (i.r. $\nu_{max.}$ 2 915, 2 860 cm^{-1}; no unsaturation) (1.45 g) which were not examined further. Fractions 9—12 (hexane–benzene, 95 : 5) gave a waxy solid (1.97 g) whose i.r. spectrum ($\nu_{max.}$ 1 730, 1 240 cm^{-1}) suggested that this material contains alkyl alkanoate esters. The n.m.r. spectrum showed signals at δ *ca.* 4.7, indicating the presence of olefinic functions.

Fractions 15—19 (hexane–benzene 9 : 1) on concentration yielded a yellowish, partly solid mixture (0.86 g), which on

[13] S. M. Kupchan, V. H. Davies, T. Fujita, M. R. Cox, and R. F. Bryan, *J. Amer. Chem. Soc.*, 1971, **93**, 4916; S. M. Kupchan, V. H. Davies, T. Fujita, M. R. Cox, R. J. Restivo, and R. F. Bryan, *J. Org. Chem.*, 1973, **38**, 1853.

[14] A. Ortega, A. Romo de Vivar, E. Diaz, and J. Romo, *Rev. Latinoamer. Quim.*, 1970, **1**, 81.

[15] A. Romo de Vivar, C. Guerrero, E. Diaz, E. A. Bratoeff, and L. Jimenez, *Phytochemistry*, 1976, **15**, 525.

[16] W. Vichnewski, S. J. Sarti, B. Gilbert, and W. Herz, *Phytochemistry*, 1976, **15**, 191.

[17] W. Vichnewski, J. N. C. Lopes, D. D. S. Filho, and W. Herz, *Phytochemistry*, 1976, **15**, 1775.

trituration and then recrystallization (6 times) from hexane gave lupeol acetate (1), (20 mg), m.p. and mixed m.p. 206—207 °C; $[\alpha]_D^{CHCl_3}$ +38° (lit.,[18] m.p. 213—215 °C, $[\alpha]_D$ +30°). The i.r. spectrum was identical with that of an authentic sample.

Fractions 25 and 26 (hexane–benzene 3 : 1) gave material which on recrystallization from hexane–benzene gave friedelin (2), m.p. 254—255 °C, $[\alpha]_D$ −19° (lit.,[19] m.p. 255—261°, $[\alpha]_D$ −29°, −28°, −21°). Identity was confirmed by mixed m.p. and i.r. spectral comparison with authentic material.

Fractions 32 and 33 (benzene) gave, after repeated recrystallization from hexane–benzene, epifriedelinol (3), m.p. 276—279 °C, $[\alpha]_D^{CHCl_3}$ +18° (lit.,[20] m.p. 282—284 °C, $[\alpha]_D^{CHCl_3}$ +15°). The acetate, prepared by refluxing the compound with acetic anhydride containing a trace of pyridine, had m.p. 290—291 °C (lit.,[21] m.p. 293—295 °C).

Fractions 35—38 (benzene) gave, after repeated crystallization from ethanol–hexane, crystalline material (4.0 g); m.p. 137—138 °C, $[\alpha]_D^{CHCl_3}$ +41.5°; i.r. $\nu_{max.}$ KBr 3 400 (O–H), 3 075, 1 640 cm^{-1} (unconjugated –C=C–); n.m.r. discussed in text.

Fractions 46—48 (benzene–chloroform 95 : 5) gave, after repeated crystallization from ethanol, crystalline material, m.p. 145—146 °C, $[\alpha]_D^{CHCl_3}$ −41.3°; i.r. $\nu_{max.}$ KBr 3 440 (OH), 3 000, and 1 635 cm^{-1} (C=C); n.m.r. δ($CDCl_3$) 5.15, 5.4 (m, *ca.* 2 H), and 3.6 (m, 1 H, H–C–OH). The mass spectrum gave two apparent molecular ions at *m/e* 414 and 412 ($C_{29}H_{50}O$ and $C_{29}H_{48}O$). The Liebermann–Burchard colour reaction was blue. These data are consonant with a sitosterol mixture.[22] Hydrogenation of this material (acetic acid, Adams catalyst, 20 °C, 1 atm H_2) gave sitostanol, m.p. 134—135 °C (lit.,[23] m.p. 137—138 °C); no olefinic signals in n.m.r. spectrum.

Chromatography of the acidic material. Chromatography of this material (9.9 g) on silica gel (Woelm, activity II—III, 700 g) employed gradient elution (hexane–ethyl acetate–methanol). Although 13 fractions, representing 98% recovery, were thus obtained, further attempted fractionation yielded no homogeneous, characterizable compounds.

Constituents of the Methanol–Water (9 : 1) *Fraction.*—(i) *The neutral fraction.* The methanol–water (9 : 1) fraction (291 g) was dissolved in chloroform and extracted successively with 5% sodium hydrogen carbonate solution and 5% sodium carbonate solution. The neutral organic phase was then evaporated to a syrup and redissolved in 95% ethanol (4 l). This solution was treated with 4% aqueous lead(II) acetate (4 l). The resulting precipitate was filtered off and washed with 50% aqueous ethanol, the washings being added to the filtrate. The ethanol was removed under reduced pressure, and the resulting oily mixture extracted with chloroform. The chloroform layer was dried ($MgSO_4$) and solvent removed under reduced pressure to give a syrup, which was dissolved in 95% ethanol. The solution was seeded with eremantholide A obtained in an earlier chromatographic work-up, and held at 3 °C for two weeks. Mixed crystalline eremantholides A and B (26.2 g) were thus obtained.

The above crystalline mixture (1 g loads) was chromatographed on silica gel in a Waters LC-500 preparative HPLC instrument, using gradient elution with either dichloromethane–methanol or dichloromethane–ethyl acetate. Six automatic recyclings separated eremantholide A (81% of the mixture) from eremantholide B (19% of the mixture). Eremantholide A had m.p. 181—183 °C, $[\alpha]_D$ +65° (EtOH); u.v. $\lambda_{max.}$ 266 nm (log ε 4.00); i.r. (KBr) $\nu_{max.}$ 3 400 (OH), 1 700 (γ-lactone), 1 695 (enone –C=O), and 1 650 and 1 593 cm^{-1} (C=C); n.m.r. described in the Discussion. Mass spectrum: M^+ 348.158 2 ($C_{19}H_{24}O_6$ requires 348.151 4). Eremantholide B had m.p. 233 °C; $[\alpha]_D$ +60° (EtOH); $\lambda_{max.}$ 266 nm (log ε 4.00); i.r. $\nu_{max.}$ 3 400 (OH), 1 770 (γ-lactone), 1 695 (enone C=O), and 1 650 and 1 590 cm^{-1} (C=C); n.m.r. δ($CDCl_3$) 6.0br (s, 1 H, 5-H), 5.55 (s, 1 H, 2-H), 4.9 (m, 1 H, 6-H), 4.0 (m, 1 H, 8-H), 2.75—3.0 (2 H, m, OH, 7-H), 1.45 (3 H, s, 14-CH_3), 1.35 (3 H, s, 13-CH_3), and 0.8—1.1 (m, 8 H, from s-butyl group). Mass spectrum: M^+ 362.172 8 ($C_{20}H_{26}O_6$ requires 362.167 1).

A portion (19 g) of the material remaining in the mother liquor was chromatographed on silica gel (activity II—III, 55 g, 4 cm × 120 cm column), employing gradient elution (hexane–benzene–chloroform–acetone). With pure chloroform, more eremantholides A and B (10.36 g) were obtained. Chloroform–acetone (3 : 1) yielded a fraction (4.03 g) containing another compound (t.l.c.); this fraction was re-chromatographed on silica gel (180 g, 2.75 × 60 cm column). Elution with chloroform and chloroform–acetone (19 : 1) gave material which after three recrystallizations from ethanol yielded pure eremantholide C (11 mg), m.p. 229—230 °C, $[\alpha]_D$ −12.7°; u.v. $\lambda_{max.}$ EtOH 266 nm (log ε 3.99); i.r. $\nu_{max.}$ 3 380, 1 760, 1 690, 1 650, and 1 580 cm^{-1}; n.m.r. spectrum discussed in the text. Mass spectrum: M^+ 346.142; $C_{19}H_{22}O_6$ requires 346.142. Despite extensive rechromatography of other fractions from these columns, no other homogeneous, characterizable compounds were obtained.

(ii) *Constituents of the hydrogen carbonate-soluble fraction.* Chromatography of this fraction (4.19 g) on silica gel (activity II—III, 250 g, 2.75 × 60 cm column) in ether gave by elution with ether tamarixetin (0.48 g), m.p. 253—256 °C; (lit.,[24] m.p. 259—260 °C; u.v. (nm, log ε in parentheses) $\lambda_{max.}$ (MeOH) 258 (4.24), 264sh (4.20), and 360 (4.22); $\lambda_{max.}$ (MeOH–$AlCl_3$) 276 (4.32), 310sh (3.79), and 4.40 (4.27); $\lambda_{max.}$ (MeOH–$AlCl_3$–HCl) 274 (4.10), 300sh (3.79), 355sh (3.93), 400 (3.99); $\lambda_{max.}$ (MeOH–NaOMe), 268 (4.06), and 370 (3.91); $\lambda_{max.}$ (MeOH–unfused NaOAc) 258 (3.90), 268 (3.88), and 362 (3.82); $\lambda_{max.}$ (MeOH–NaOAc–H_3BO_3) 270 (4.56) and 358 (3.58). The n.m.r. spectrum was in accord with that published for the trimethylsilyl ether of tamarixetin.[25]

(iii) *Constituents of the sodium carbonate-soluble fraction.* This extract (4.44 g) was chromatographed on silica gel (silica AR CC-4, 200 g, 2.75 × 50 cm). Elution with benzene–ether (3 : 1) gave two compounds; that eluted earlier was crystallized from methanol and was identified as genkwanin (8 mg); m.p. 280—283 °C (lit.,[26] m.p. 285—287 °C); u.v. $\lambda_{max.}$ (MeOH) 268 (3.99) and 338 (4.12); $\lambda_{max.}$ (MeOH–$AlCl_3$), 278 (4.06), 300 (3.99), 342 (4.11), 380 (3.96);

[18] R. O. Lindgren and C. M. Svahn, *Acta Chem. Scand.*, 1966, **20**, 1720.
[19] T. Bruun, *Acta Chem. Scand.*, 1954, **8**, 71.
[20] W. H. Hui and C. T. Ho, *Austral. J. Chem.*, 1968, **21**, 1675.
[21] V. Anjeneyulu, D. N. Rao, and L. R. Row, *J. Indian Chem. Soc.*, 1967, **44**, 123.
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[26] Ref. 25, p. 148.

$\lambda_{max.}$ ($AlCl_3$–HCl), 276 (4.03), 300 (3.98), 340 (4.08), and 384 (3.93); $\lambda_{max.}$ (NaOMe) 265, 298sh, and 388; $\lambda_{max.}$ (MeOH–unfused NaOAc), 270 (4.09), and 340 (4.09); $\lambda_{max.}$ (MeOH–NaOAc–H_3BO_3), 270 (4.11), and 340 (4.11); n.m.r. δ(Polysol) 3.84 (s, 3 H, OCH_3), 6.4 (1 H, d, 2 Hz, 6-H or 8-H), 6.6 (1 H, d, 2 Hz, 8-H or 6-H), 6.62 (s, 1 H, 3-H), 6.9 (d, part of AB system, 2 H, 3′-H and 5′-H), 7.8 (d, part of AB system, 2 H, 2′-H and 6′-H). The latter compound eluted was luteolin 7-methyl ether, m.p. 266—268 °C (lit.,[27] m.p. 260—262 °C); u.v. $\lambda_{max.}$ (EtOH), 256 (4.28), 266 (4.07), 350 (4.18); $\lambda_{max.}$ (EtOH–$AlCl_3$), 272 (4.08), 293 (3.83), 355 (3.99), and 390 (4.05); $\lambda_{max.}$ (EtOH–$AlCl_3$–HCl), 272 (4.12), 294 (3.95), 355 (4.11), and 385 (4.12); $\lambda_{max.}$ (EtOH–NaOEt) 265 (4.02) and 405 (4.18); $\lambda_{max.}$ (EtOH–unfused NaOAc), 257 (3.89), 267 (3.88), and 354 (3.85); $\lambda_{max.}$ (EtOH–NaOAc–H_3BO_3), 262 (3.80) and 365 (3.88), n.m.r. δ(Polysol) 3.8 (s, 3 H, OCH_3), 6.3 and 6.5 (2 H, J = 2 Hz, 6-H and 8-H), 6.5 (s, 1 H, 3-H), 6.9 (1 H, m, 5′-H), and 7.4 (2 H, m, 2′,6′-H).

Addition of Propane-1-Thiol to Eremantholide A. (cf. Ref. 28).—Propane-1-thiol (0.5 ml) was added to a solution of eremantholide A (50 mg) in tetrahydrofuran (1.35 ml) and pH 9.2 borate buffer (0.9 ml). The reaction mixture was held at 20 °C for 15—20 days being monitored by t.l.c. until starting material had virtually disappeared. Water was then added and the reaction mixture worked up *via* ether. The product crystallized from ethanol as needles (50%) m.p. 236—241 °C; spectral details are given in the Discussion.

This work was supported by a grant (CA 13001-02/04) awarded by the National Cancer Institute, National Institutes of Health. Plant material used in this investigation was collected (November 1972) in the state of Minas Gerais, Brazil, by Dr. A. Pereira-Duarte (No. 12179). A voucher specimen has been deposited in the herbarium (at Belo Horizonte).

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[8/411 *Received*, 6*th March*, 1978]

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