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SESQUITERPENE LACTONES FROM *PODANTHUS OVATIFOLIUS**

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Key Word Index—*Podanthus ovatifolius*; Compositae; sesquiterpene lactones; cytotoxicity; anticancer agents; ovatifolin; erioflorin derivatives; structure determination and correlation.

Abstract—The title plant has been shown to contain the sesquiterpene lactones erioflorin methacrylate (I), erioflorin (III), erioflorin acetate (VIII) and a new lactone, ovatifolin (X).

INTRODUCTION

IN A ROUTINE screening programme of Chilean flora for anticancer drugs,[1] it was found that an alcoholic extract of the plant *Podanthus ovatifolius* Lag. (Compositae: tribe Heliantheae), gave a positive test in the standard KB and PS assay procedures.[2] Since members of the Compositae often contain sesquiterpenes and since sesquiterpene lactones sometimes show activity in the anti-tumour assays,[3] a study of the neutral fractions from this plant was initiated.

RESULTS AND DISCUSSION

A total of six sesquiterpene fractions have been isolated to date from the neutral extracts of *P. ovatifolius*, of which three represent new natural products. By far the major component proved to be erioflorin methacrylate (I) (0·12%). This component had m.p. 155–158° and analysed as $C_{23}H_{28}O_7$. Its IR spectrum showed a γ-lactone at 1760 cm^{-1}, a strong unsaturated ester band at 1715 cm^{-1}, and strong carbon–carbon double bond absorptions (1666 and 1630 cm^{-1}). Its UV spectrum showed the presence of three unsaturated ester and lactone groups (λ_{max} 211 nm, ϵ 24 700). That the α-methylene-γ-lactone function was present was also indicated by the strong IR absorption at 960 cm^{-1}.

The MS of this ester showed a parent ion at *m/e* 416, consistent with the assigned formula. It also showed, however, a small peak at *m/e* 430, Repeated TLC separation and crystallization failed to completely eradicate the presence of the 430 peak and it has therefore been

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[1] For Part 1 see BITTNER, M., POYSER, K. A., POYSER, J. P., SILVA, M., WELDT, E. and SAMMES, P. G. (1973) *Phytochemistry* **12**, 1427.

[2] Cytotoxicity (KB) and *in vivo* (PS) tests were carried out at the National Cancer Institute, N.I.H. For procedures see *Cancer Chemother. Rep.* **25**, 1 (1962).

[3] For examples see KUPCHAN, S. M., EAKIN, M. A. and THOMAS, A. M. (1971) *J. Med. Chem.* **14**, 1147.

ascribed to the co-occurrence of traces of the monoangelate (or tiglate) ester. The presence of the two methacrylyl residues in the sesquiterpene was shown by the sequential loss of two methacrylic acid units, giving rise to strong peaks at *m/e* 330 and 244 respectively.

TABLE 1. NMR PEAKS OF ISOLATED SESQUITERPENES AND DERIVATIVES*

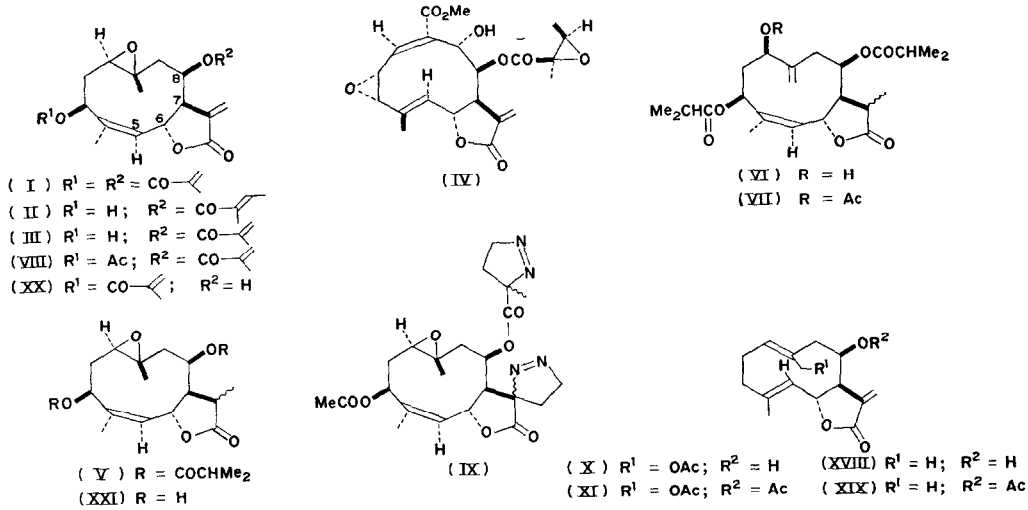
Compound	(I)†	(V)†	(VI)	(VII)	(VIII)	(X)†	(XI)†
C_4–Me	8·10*d* (1·5)	8·08*d* (1·25)	8·22*d* (1·5)	8·18*d* (1·5)	8,12*d* (2·0)	8·30*d* (1·3)	8·34*d* (1·3)
C_{10}–Me	8·60*s*	8·48*s*	4·69, 4·91‡	4·58, 4·80‡	8·55*s*	5·30, 5·5§ (12)	5·32, 5·75§ (12)
C_{11}–CH_2	4·28*d* (2·0) 3·80*d* (2·0)	8·90*d*‖ (7)	—¶	—	4·32*d* (2·0) 3·68*d* (2·0)	4·48*d* (3·0) 3·70*d* (3·5)	4·48*d* (3·0) 3·77*d* (3·5)
H_1	7·30*dd* (4, 10)	7·25*dd* (4, 10)	—	—	7·38*dd* (4, 10)	4·8*m*	4·8*m*
H_2	8·22*ddd* (2, 10, 14·5) 7·50*ddd* (4, 5, 14·5)	8·30*ddd* (2, 10, 15) 7·30*ddd* (4, 6, 15)	—	—	8·25*m* 7·53*m*	—	—
H_3	4·80*dd* (2, 5)	4·85*dd* (2, 6)	—	—	*ca.* 4·8**	—	—
H_5	4·80*dq* (11, 1·5)	4·58*dq* (12, 1·2)	4·60*m*	4·55*m*	*ca.* 4·8**	5·27*bd* (10·5)	5·22*dq* (10, 1·3)
H_6	3·80*dd* (2, 11)	4·18*dd* (0·5, 12)	4·30*m*	4·23*m*	3·89*dd* (2, 11)	4·87*dd* (9, 10·5)	4·90*bdd* (8·5, 10)
H_7	7·10*m*	*ca.* 7·5**	—	—	7·2**	7·3*m*	7·2*m*
H_8	4·85*m* (4·5, 2, rest small)	4·95*m*	—	—	*ca.* 4·8**	5·5*m*	4·3*m* (5, rest small)
H_9	7·2*dd* (4·5, 15·5) 8·60*dd* (2, 15)	7·30*dd* (4·5, 15) 8·76*dd* (1·5, 15)	—	—	*ca.* 7·2** 8·6*dd* (2, 15)	—	7·7 6·88*dd* (5, 14)
Others	Methacrylates: 8·14, 8·06 (Me) 4·68, 4·46, 4·02, 3·95 (vinyl H)		8·8–9·0 (5 Me)	8·0 (acetate)	7·9 (acetate)	8·0 (acetate)	8·0 (acetates)

* Carried out at 100 MHz with $CDCl_3$ as solvent; *s*, *d*, *q*, *m* and *b* have normal meanings. Chemical shifts in τ values, coupling constants in Hz.
† $Eu(fod)_3$ and decoupling experiments used.
‡ Vinyl methylene.
§ AB quartet.
‖ Reduced, present as methyl group.
¶ Blank spaces means no satisfactory shift could be assigned since the peak is part of a complex pattern.
** Buried in complex pattern.

The initial structural assignment of the sesquiterpene nucleus was largely based on NMR studies. Extensive decoupling experiments indicated the part structures (a), (b) and (c). With the aid of europium induced shift experiments,[4] using the $Eu(fod)_3$ reagent, a complete analysis of all the protons in the system was achieved (Table 1). From the coupling constants

[4] RONDEAU, R. E. and SIEVERS, R. E. (1971) *J. Am. Chem. Soc.* **93**, 1522.

observed important relative stereochemical assignments could be made. Thus, the coupling constants across the protons at positions 5–7 showed $J_{5,6}$ 11 Hz, and $J_{6,7}$ 2 Hz, equivalent to the values found in heliangine (II)[5], the structure of which has been confirmed by an X-ray crystallographic analysis.[6] The latter sesquiterpene has the 4,5-bond *cis*-oriented and the lactone group *trans*-fused. That the 4,5-bond was *cis*-oriented in the erioflorin methacrylate isolated was further supported by hydrogenation experiments. Reduction over platinum gave absorption of three equivalents of hydrogen, the nuclear double bond remaining unreduced since, in the *cis*-configuration, it lies orthogonal to the general plane of the heliangolide ring,* hindering reduction. Similar results were found both with heliangine (II) and with erioflorin (III).[8]



An important consequence of the *cis*-4(5) bond is its effect on the conformation about the lactone ring. In its most favourable conformation (as evinced by Dreiding models and by the solid state conformation of heliangine monochloroacetate[6]) the lactone junction is twisted so that the dihedral angle between H-6 and H-7 is decreased (to *ca.* 105°) compared to that for compounds with a *trans*-4(5) bond [e.g. melampodin (IV), *ca.* 160°]. As a consequence the coupling constant across the 6,7-bond is small in the former case (*ca.* 2 Hz), compared to that in the latter systems (*ca.* 10 Hz). This effect may be of some diagnostic value in the assignment of certain germacradienolides to their subgroups. Some examples are quoted in Tables 2 and 3.

The orientation of the 8-methacryloyl substituent as β was also consistent with the observed NMR coupling constant of 2 Hz between positions 7 and 8. The relative stereochemistry of the remaining epoxide and ester functions was finally achieved by the appro-

* In a recent communication[7] germacradienolides having the Δ^4 bond *cis* and the $\Delta^{1(10)}$ bond *trans* have been subgrouped as 'heliangolides'.

[5] Morimoto, H., Sanno, Y. and Oshio, H. (1966) *Tetrahedron* **22**, 3173.
[6] Nishikawa Kamiya, M., Takabatake, A., Oshio, H., Tomiie, Y. and Nitta, I. (1966) *Tetrahedron* **22**, 3601.
[7] Rogers, D., Moss, G. P. and Neidle, S. (1972) *J.C.S. Chem. Commun.* 142.
[8] Torrance, S. J., Geissman, T. A. and Chedekel, M. R. (1969) *Phytochemistry* **8**, 2381.

priate esterification of an authentic sample of erioflorin with methacrylyl chloride,[9] which was remarkably slow (after 3 days only a low yield of the ester had formed). The authentic and natural samples were identical. Heliangine methacrylate was also prepared from an authentic sample of heliangine but we were unable to detect any of this compound in the extracts from *P. ovatifolius*.

TABLE 2. SELECTED NMR PARAMETERS, ERIOFLORIN AND ANALOGUES WITH *cis*-4,5 DOUBLE BONDS* (AND *trans*-FUSED LACTONE)

Compound	C_{11}–CH_2	C_4–Me	H_5	H_6	H_7	H_8
Erioflorin methacrylate (I)	4·28*d* (2·0) 3·80*d* (2·0)	8·10*d* (1·5)	3·80*dd* (1·5, 11)	3·80*dd* (2, 11)	7·10*m*	4·85*m* (4.5, 2, rest small)
Erioflorin (III)[8]	4·17*d* (2·0) 3·65*d* (2·0)	8·16*d* (0·5)	4·69*bd* (11)	3·5*dd* (1, 11)	—	—
Heliangine (II)[5]	4·3*d* (2·0) 3·7*d* (2·0)	8·1*bs*	4·7*d* (11)	3·5*d* (11)	—	5·72*m*
Dihydroheliangінyl diisobutyrate (V)	8·90†	8·08*d* (1·25)	4·58*dq* (1·2, 12)	4·18*dd* (0·5, 12)	7.5*m*	4·95*m*
Melampodine (IV)[23]	4·40*d* (3·0) 3·81*d* (3·0)	7·90*bs*	4·6–4·9*d* (10)	4·6–4·9*dd* (10, 10)	7·42*m*	3·79*dd* (8·5, 1·5)

* Recorded for solutions in $CDCl_3$.
† Methyl doublet, *J ca.* 7 Hz.
‡ *trans*-4,5 bond; added for comparison.

Attempted hydrolysis of erioflorin methacrylate under mild basic conditions (potassium carbonate in methanol) afforded a complex mixture of products (at least 8 compounds) in agreement with the results obtained for both heliangine[5] and erioflorin.[8] Not all of these hydrolysis products were completely characterized, however, all of the major components exhibited methoxy resonances in their NMR spectra due to addition across the unsaturated lactone group (see Experimental). Attempted hydrolysis of the hexahydro-erioflorin methacrylate (V) also afforded a complex mixture, but a small sample of 11,13-dihydroheliangінol was isolated (see Experimental). In contrast, acid-catalyzed opening of the epoxide ring[8] occurred smoothly to give one major product, the alcohol (VI), which was also characterized as the derived acetate (VII).

The second compound isolated from the plant, in the benzene-ethyl acetate fraction, proved to be the acetate of erioflorin (VIII) (0·02%), shown to be identical to an authentic sample. This acetate ester had been prepared from erioflorin but it has not previously been isolated from a plant (e.g. *Eriophyllum confertiflorum*[8]). Reduction of the natural material over platinum oxide afforded a tetrahydro-derivative which was also identical to a sample prepared from the authentic erioflorin (III). Treatment of erioflorin acetate with an excess of diazomethane afforded one major, bis-pyrazoline (IX).

[9] HAWORTH, W. N., GREGORY, H. and WIGGINS, L. F. (1946) *J. Chem. Soc.* 488.

The extract also contained a mixture of erioflorin (III) and a new lactone, ovatifolin, which could not be separated by chromatographic techniques. This mixture was best separated as the derived acetates. It was eventually found that the second, unknown component could be isolated by repeated fractional crystallization of the mixture. The NMR spectrum of the pure compound was identical to that of the mixture, after subtraction of the peaks due to erioflorin. The new compound was named ovatifolin (X). Examination of the derived acetates from the mixture confirmed the presence of erioflorin.

TABLE 3. SELECTED NMR PARAMETERS, OVATIFOLIN AND ANALOGUES*

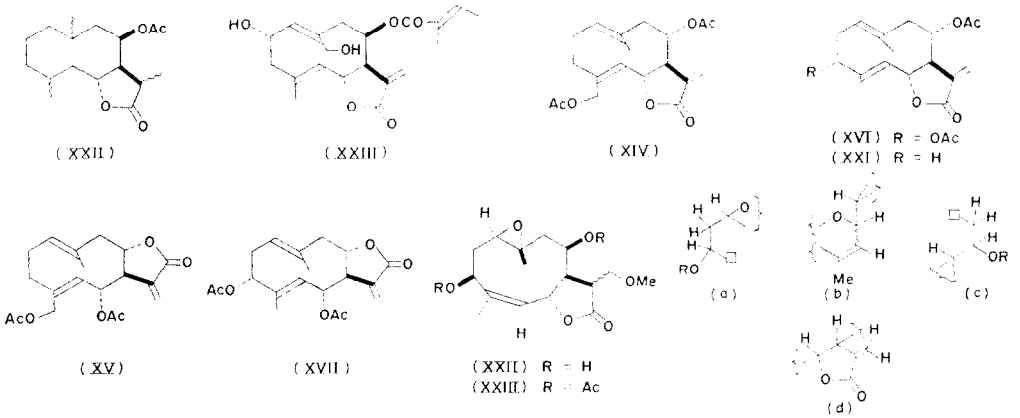
Compound	C_{11}–CH_2	C_4–Me	H_5	H_6	H_7	H_8
Ovatifolin acetate (II)	4·48*d* (3·0) 3·77*d* (3·5)	8·34*d* (1·3)	5,22*dq* (1·3, 10)	4·90*bdd* (10, 8·5)	7·16*m*	4·33*m*
Epitulipinolide (XIX)[16]	4·41*d* (3·0) 3·72*d* (3·5)	8·24*d* (1·3)	5·22*dq* (1·3, 10)	4·87*dd* (10, 8·1)	7·07*m*	4·28*m*
Tulipinolide (XXI)[16]	4·16*dd* (1·5, 3·0) 3·66*dd* (1·5, 3·5)	8·29*bs*	4·8–5·2	4·8–5·2	6·92*m*	4·8–5·2
Chihuahuin (XVI)[13]	4·21*dd* (1·0, 3·0) 3·70*dd* (1·2, 3·5)	8·37*d* (1·5)	4,63*bd* (10)	5·05*dd* (8, 10)	6·90*m*	5·08*m*
Ovatifolin (X)	4·48*d* (3·0) 3·70*d* (3·5)	8·30*d* (1·3)	5·27*bd* (10·5)	4·86*dd* (9, 10·5)	7·3*m*	5·5*m*
Eupatolide (XVIII)[15]	4·29*d* (3·0) 3·48*d* (3·0)	8·30*d* (1·5)	5·15*m*	4·31*dd* (7·5, 10)	7·2*m*	5·4*m*

* Recorded for solutions in $CDCl_3$, except for (XVIII), recorded in D_5-pyridine.

Ovatifolin acetate (XI) analyzed as $C_{19}H_{24}O_6$ and showed IR bands for ester and lactone groups (1739, 1765 cm^{-1}) as well as the typical absorptions of the α-methylene-lactone group (1660 and 970 cm^{-1}). Its NMR spectrum showed the presence of two acetate peaks and one vinyl methyl group as well as the presence of two vinyl protons other than those due to the methylene group (see Table 1). Again europium shift experiments were helpful in clarifying the decoupling work. From these experiments the part structures (b), (c) and (d) were established. Hydrogenation afforded one major product, a deacetoxy-hexahydroderivative (XII). Loss of one of the acetoxy groups by hydrogenolysis suggested the presence of an allylic acetate function, as occurs for eriofertin (XIII).[10] That this was originally present on a vinylic methylene group was confirmed by the appearance of a new, secondary methyl group in the NMR spectrum of the reduction product.

[10] SAITOH, T., GEISSMAN, T. A., WADDELL, T. G., HERZ, W. and BHAT, S. V. (1971) *Revista Latinamer. Quim.* **2**, 69.

Several sesquiterpene lactone isomers of ovatifolin acetate are known. These include salonitenolide acetate (XIV),[11] artemisiifolin acetate (XV),[12] chihuahuin (XVI),[13] and chamissonin acetate (XVII)[14] but none of these had NMR properties consistent with those of ovatifolin acetate. Recently zexbrevin-*D* has been isolated from the plant *Zexmenia brevifolia* and it has been assigned the structure (XI),* but with the 8ζ-ester function. A direct comparison with ovatifolin acetate showed that they had identical properties. The site of the acetate function in ovatifolin itself was resolved by an examination of its NMR spectrum (Table 1); only a single methine proton moves markedly downfield upon acetylation. The configuration about the 4(5)-bond was consistent with a *trans*-orientation.



A comparison of the NMR parameters (Table 3) of ovatifolin (X) with eupatolide (XVIII)[15] and of the acetate (XI) with epitulipinolide (XIX)[16] indicates their similarity about centres 5–8. That the alcohol function at position 8 is β follows from this comparison and from the lack of coupling of the C-13 methylene protons with each other[17] (J_{gem} *ca.* 1 Hz). An attempt was therefore made to correlate the structure of ovatifolin acetate (and zexbrevin D) with epitulipinolide (XIX). Provided the steric course of reduction of the double bonds of both ovatifolin acetate and of epitulipinolide take the same course the structure of the major reduction production from each should be identical. In the event, both afforded similar products. (XII), undepressed on mixed melting point and with identical TLC properties but which showed slight differences in their NMR and IR spectra and so a definite correlation between the two series cannot be claimed at this point. It is possible, however, that the minor differences of the two samples were due to the presence of minor amounts of stereoisomers, although these were not apparent from the TLC examination.

* We thank Professor J. Romo, Instituto de Quimica, Universidad Nacional Autonoma de Mexico for this information prior to publication.

[11] YOSHIOKA, H., RENOLD, W. and MABRY, T. J. (1970) *Chem. Commun.* 148.
[13] RENOLD, W., YOSHIOKA, H. and MABRY, T. J. (1970) *J. Org. Chem.* **35**, 4264.
[12] PORTER, T., MABRY, T. J., YOSHIOKA, H. and FISCHER, N. H. (1970) *Phytochemistry* **9**, 199.
[14] L'HOMME, M. F., GEISSMAN, T. A., YOSHIOKA, H., PORTER, T. H., RENOLD, W. and MABRY, T. J. (1969) *Tetrahedron Letters* 3161.
[15] LEE, K.-H., HUANG, H.-C., HUANG, E.-S. and FURUKAWA, H. (1972) *J. Pharm. Sci.* **61**, 629.
[16] DOSKOTCH, R. W. and EL-FERALY, F. S. (1970) *J. Org. Chem.* **35**, 1928.
[17] See comment in Ref. 10, p. 69.

The correlation of erioflorin and its derivatives with heliangine, as mentioned above, originally suggested to us that the sesquiteroene components from *P. ovatifolius* should belong to the heliangolide sub-group,[7] and hence that ovatifolin would also bear a *cis*-4(5) double bond. The evidence cited above suggests that this bond is *trans*. It is of interest to note, therefore, that all examples of the 'heliangolide' sesquiterpenes[7] so far isolated contain the 1(10)-expoxide group and are oxygenated at position 3, generally as a 3β-hydroxyl group. This might suggest that the *cis*-4(5)-bond arises from a *trans*-oriented precursor after (or during) the oxidation which introduces these functions.

Several other germacranolide sesquiterpene lactones containing a *cis*-4,5 double bond are known, including woodhousin,[18] eupacunin,[19] liatrin,[20] calaxin,[21] ciliarin,[21] and orizabin,[22] but in all these cases some ambiguity arises as to the origin of the substituents about positions 1 and 10 and, since no double bond is present at the latter site, they cannot be classified according to the scheme of Rogers *et al.*[7]

Two minor sesquiterpene components were also isolated from the plant and further work on these is in progress. The results of the antitumour tests with the compounds (I), (VIII), and (X) were of interest. Whereas the acetate (VIII) and ovatifolin (X) were active in both the P388 mouse leukaemia and the KB human epidermoid cancer tests, the methacrylate (I) was only active in the former test.

EXPERIMENTAL

M.ps were taken on a Kofler block and are uncorrected. UV spectra were recorded in EtOH and IR spectra as Nujol mulls. Rotations were taken at 20–25° and NMR spectra were recorded in $CDCl_3$ with tetramethylsilane as internal reference. MS were obtained with an AEI MS9 double focussing instrument, Merck silica gel was used for TLC and PLC.

Extraction of the plant. The powdered, dry leaves and stems of *P. ovatifolius* (6 kg), collected in Rocoto, Chile in summer, were extracted with EtOH, the solvent removed *in vacuo* and the gummy residue macerated with H_2O. The precipitated material (400 g) was collected, redissolved in EtOH and H_2O added until precipitation just began. The solution was extracted with light petrol. (60–80°), followed by EtOAc–C_6H_6 (1 : 9) (to give 47 g), and EtOAc (to give 28 g). The preferred isolation of compounds from the initial extracts was by direct PLC, initially with EtOAc–C_6H_6 (1 : 4), and then as indicated, followed by crystallization. The R_f value quoted after the first mention of each compound refers to the above solvent system on SiO_2 GF_{254}.

Erioflorin methacrylate (1) (R_f 0·6). Crystallization of the major component from the initial chromatography afforded *erioflorin methacrylate* (1) (0·12%), m.p. 155–158° (hexane–EtOAc), $[\alpha]_D$ −87° (*c* 1·0, $CHCl_3$), ν_{max} 1760, 1715, 1666, 1630, 1160, 1130 cm^{-1}, λ_{max} 211 nm (ε 24 700), NMR, see Table 1, *m/e* 416 (M^+), 347 (M^+-methacrylyl), 330 (M^+-methacrylic acid), 261, 240 (M^+-2 methacrylic acid units), 83, 69, 55 (Found: C, 66·15; H, 6·89. $C_{23}H_{28}O_7$ requires: C, 66·33; H, 6·78%). All samples of the material also showed a very small peak at *m/e* 430 to their MS, assigned to the presence of traces of a homologous ester (angelate or tiglate). A synthetic sample of erioflorin methacrylate was prepared by treatment of an authentic sample of erioflorin[15] with methacrylyl chloride[9] in pyridine for several days. Isolation, in the normal manner, followed by PLC isolation and crystallization from hexane–EtOAc gave material m.p. 156–158°, m.m.p. 154–156°, $[\alpha]_D$ −67° (*c* 0·5, EtOAc), identical by IR and NMR spectral comparison and by extensive TLC and MS comparisons.

Heliangine methacrylate. Treatment of an authentic sample of heliangine (2) (130 mg) with an excess of methacrylyl chloride in pyridine for 2 days gave, after the normal extraction and PLC, the *methacrylate ester* (21 mg, 14%), m.p. 147–149° (EtOAc–hexane), $[\alpha]_D$ −97° (*c* 0·5, EtOAc), ν_{max} 1763, 1713, 1670, 1651, 1640, 1277, 1268, 1164, 1138, 1110 and 972 cm^{-1}, λ_{max} 212 nm (ε 33 000), M^+ 430 (weak) (Found: C, 66·7; H, 7·1. $C_{24}H_{30}O_7$ requires: C, 66·7; H, 7·0%).

[18] HERZ, W. and BHAT, S. V. (1972) *J. Org. Chem.* **37**, 906.
[19] KUPCHAN, S. M., MARUYAMA, M., HEMINGWAY, R. J., HEMINGWAY, J. C., SHIBUYA, S., FUJITA, T., CRADWICK, P. D., HARDY, A. D. U. and SIM, G. A. (1971) *J. Am. Chem. Soc.* **93**, 4914.
[20] KUPCHAN, S. M., DAVIES, V. H., FUJITA, T., COX, M. R. and BRYAN, R. F. (1971) *J. Am. Chem. Soc.* **93**, 4916.
[21] ORTEGA, A., ROMO DE VIVAR, A., DIAZ, E. and ROMO, J. (1970) *Rev. Latinamer. Quim.* **1**, 82.
[22] ORTEGA, A., GUERRERO, C., ROMO DE VIVAR, A., ROMO, J. and PALAFOX, A. (1971) *Rev. Latinamer. Quim.* **2**, 38.
[23] FISCHER, N. H., WILEY, R. and WANDER, J. D. (1972) *J. C. S. Chem. Commun.* 137.

Reduction of erioflorin methacrylate. Erioflorin methacrylate (55 mg) was shaken with PtO_2 (10 mg) in EtOH (10 ml) under H_2 at room temp. and pressure for 4 hr during which time 3 mol of H_2 were absorbed. Filtration and evaporation afforded needles of 11,13-*dihydroheliangingyl* 3β,8β-*diisobutyrate* (5) (51 mg, 89%), m.p. 161–162° (hexane–Et_2O), $[\alpha]_D$ −68° (*c* 1·1, $CHCl_3$), ν_{max} 1772, 1738, 1727, 1678, 1189, 1162 and 969 cm^{-1}, λ_{max} 205 nm (ε 4900), NMR, see Table 1, *m/e* 422 (M^+), 407, 351 (M^+-isobutyroyl), 334 (M^+-isobutyric acid), 263 (base peak, M^+-isobutyroyl-isobutyric acid), 246, 137, 95, 71 (Found: C, 65·17; H, 7·94. $C_{23}H_{34}O_7$ requires: C, 65·38; H, 8·11%).

Hydrolysis of erioflorin methacrylate. The ester (620 mg) was treated with 0·3% w/v aq. K_2CO_3 (12 ml) in MeOH (120 ml) for 3·5 hr before concentration under reduced pressure, extraction with EtOAc, washing with 2N HCl and H_2O, before drying (Na_2SO_4) and evaporating to small vol. TLC showed the presence of 8 compounds of which four were isolated, using preparative TLC, The least polar product (45 mg) crystallized from EtOAc, m.p. 217–219°, $[\alpha]_D$ −48° (*c* 1·1, $CHCl_3$), ν_{max} 1761 (lactone), 1717, 1675, 1302, 1232, 1195, 1163, 1133 cm^{-1}, λ_{max} 210 nm (ε 16 000); its MS showed it to be a mixture of the methoxymethyl lactones produced by addition of MeOH across the unsaturated lactone ring-traces of the angelate (tiglate) ester impurity were also present, viz *m/e* 462 (M^+ of angelate ester, MeOH adduct), 448 (M^+ of methacrylate ester adduct), 379, 362, 307, 293, 276, 261, 173, 83 (base peak) and 69 (Found: C, 64·89; H, 7·41. $C_{24}H_{32}O_8$ requires: C, 62·27; H, 7·19 (MW 448); $C_{24}H_{32}O_8$ requires: C, 64·92; H, 7·41 (MW 462). Its NMR spectrum confirmed the addition of 1 mol MEOH, showing a methoxyl resonance at τ 6·68 (3H, *s*). The second, more polar fraction (55 mg) appeared to be *mono-deacylated compound*; on recrystallization the small methoxyl resonance, present in the crude fraction, disappeared. This fraction, however, was not identical to erioflorin. It had m.p. 189–191°, $[\alpha]_D^{25}$ −81° (*c* 0·9, $CHCl_3$), ν_{max}^{Nujol} 3468, 1753, 1715, 1657, 1639, 1274, 1160, 1137, 973 cm^{-1}, λ_{max} 211 (ε 11 200); τ 3·69 (1H, *d*, *J* 2 Hz), 4·37 (1H, *d*, *J* 2 Hz), 3·89 (1H, *bs*), 4·01 (1H, *d*, *J* 2 Hz), 4·45 (1H, narrow *m*), 4·75 (1H, *dd*, *J* 1·5, 11 Hz), 4·74 (1H, *dd*, *J* 2, 4 Hz), 5·94 (1H, *bs*), 7·1–7·6 (5H), 8·06 (3H, *bs*), 8·14 (3H, *d*, *J* 1·5 Hz), 8·49 (3H, *s*), 8,69 (1H, *bs*), 8·82 (2H, *m*), *m/e* 348 (M^+), 294, 279, 262, 123, 83, 69 (base peak), 55, 41 (Found: C, 65·43; H, 7·17. $C_{19}H_{24}O_6$ requires: C, 65·50; H, 6·94). This fraction has been assigned the structure (XX). The third fraction, more polar than the other two, was not completely characterized. It had m.p. 163–166° (hexane-ethyl acetate), $[\alpha]_D$ −46° (*c* 0·9, ethyl acetate). The most polar fraction investigated from the hydrolysis proved to be 11,13-dihydro-13-methoxyhelianginol (XXII) (66 mg), non-crystalline, ν_{max} 3430, 1760, 1735, 1670, 1210, 1100, 983, 956, 937 and 732 cm^{-1}. This was characterized as its *diacetyl derivative* (XXIII); Ac_2O-pyridine), m.p. 206–208° (C_6H_6–EtOAc), $[\alpha]_D$ −105° (*c* 0·4, $CHCl_3$), ν_{max} 1757, 1742, 1736, 1239, 1250, 1220, 1197, 1134, 1030, 988, 975 cm^{-1}; λ_{max} 205 nm (ε 3300); τ 4·07 (1H, *dd*, *J* 2, 11 Hz), 4·51 (1H, *d*, *J* 11 Hz), 4·8–5·0 (2H, *m*), 5·85 (1H, *d*, *J* 5·5 Hz), 6·42 (2H, *m*), 6·70 (3H, *s*), 7·93 (3H, *s*), 7·95 (3H, *s*), 8·14 (3H, *d*, *J* 1 Hz), 8·55 (3H, *s*), 8·78–9·12 (methylenes); *m/e* 396 (M^+), 381, 354, 353, 336, 293, 276, 261, 244, 231, 191, 173, 135, 95, 69, 43 (base peak) (Found: C, 61·06; H, 6·88. $C_{20}H_{28}O_8$ requires: C, 60·59; H, 7·12%).

Hydrolysis of reduced erioflorin methacrylate. Using the conditions employed for erioflorin methacrylate, the reduction product (V) (360 mg) was treated with methanolic K_2CO_3. A complex mixture of products was formed but one of these had similar properties to 11,13-dihydroheliangingol (XXI).[5]

Acid treatment of reduced erioflorin methacrylate. The ester (5) (198 mg) was treated with 0·05 ml conc. HCl in acetone (20 ml) at reflux for 6 hr. Extraction in the normal manner[8] and PLC (EtOAc–C_6H_6, 3:7) afforded the *alcohol* (VI) (92 mg; 70%) as needles, m.p. 119–121° (Et_2O–hexane), $[\alpha]_D$ −2° (*c* 1·25, $CHCl_3$), ν_{max} 3537, 3091, 1770, 1722, 1718, 1680, 1646, 1200, 1193, 1165, and 995 cm^{-1}; λ_{max} 206 nm (ε 4600); NMR (see Table 1); *m/e* 422 (M^+), 405, 352, 334, 264, 246, 173, 71 (Found: C, 65·39; H, 7·87; $C_{23}H_{34}O_7$ requires: C, 65·38; H, 8·11%). Acetylation of the alcohol (VI) with Ac_2O in pyridine at room temp. gave the *acetate* (VII), m.p. 185–186° (hexane–EtOAc), $[\alpha]_D$ +56° (*c* 1·6, EtOAc), ν_{max} 3100, 1771, 1736, 1729, 1720, 1670, 1650, 1238, 1191, 1182, 1160, 1019, 977, 934, 906 cm^{-1}; NMR (see Table 1); *m/e* 464 (M^+), 405, 393, 377, 334, 333, 246, 173, 71 (base peak) (Found: C, 64·77; H, 7·80. $C_{25}H_{36}O_8$ requires: C, 64·63; H, 7·81%).

Reaction of erioflorin methacrylate with diazomethane. Treatment of the natural product, in ether, with an excess of diazomethane in Et_2O overnight at 5° gave a mixture of pyrazoline adducts which were not separated by PLC. The major fraction showed m.p. 95–99° (dec.), ν_{max} 1770 and 1736 cm^{-1}, λ_{max} 208 and 323 nm (ε 8500 and 750); *m/e* 514 (weak, M^+-N_2), 486, 458, 443, 375, 358, 290, 275, 258 (Found: 375·1816. $C_{21}H_{27}O_6$ requires: 375·1808).

Erioflorin acetate (VIII) (R_f 0·55). Rechromatography of this fraction on SiO_2 (4×, EtOAc–C_6H_6, 1:15) and isolation afforded the acetate (IX) (0·02%), m.p. 193–196° (EtOAc–hexane) (lit.[8] 210–212°), $[\alpha]_D$ −114° (*c* 1·5, $CHCl_3$), ν_{max} 1766, 1733, 1720, 1670, 1634, 1250, 1162 cm^{-1}, λ_{max} 208 nm (ε 15 300), *m/e* 390 (M^+), 330, 321, 304, 261, 244, 83, 69 and 55 (Found: C, 64·93; H, 6·74; $C_{21}H_{26}O_7$ requires; C, 64·62; H, 6·67%). A sample of erioflorin acetate prepared from a reference specimen of erioflorin had m.p. 201–204°, m.m.p. 196–198° with identical spectral and chromatographic behaviour. Erioflorin acetate was also isolated from the erioflorin-ovatifolin fraction of the extraction after prior acetylation. For NMR parameters see Table 1.

Bis-pyrazoline of erioflorin acetate. Treatment of erioflorin acetate (45 mg) with CH_2N_2 in Et_2O–MeOH for 12 hr, followed by PLC (EtOAc–C_6H_6, 1:4) afforded one major product which crystallized from hexane–EtOAc as the *bis-purazoline* (IX), m.p. 131–132° (dec.), ν_{max} 1774, 1747, 1735, 1550, 1240, 1216, 1164, 1132,

1035, 1027 cm^{-1}; τ 3·49 (1H, *dd*, *J* 1·2, 12 Hz), 3·77 (1H, *dd*, *J* 1·2, 11 Hz), 4·72 (1H, *m*), 4·87(1 H, *m*), 5·31 (1H, *bs*), 5·40 (1H, *s*), 5·47 (1H, *s*), 7·48 (2H, broadened *s*), 7·82 (3H, *s*), 8·03 (3H, *d*, *J* 1·2 Hz), 8·45 (3H, *s*), 8·48 (3H, *s*); *m/e* 418 (M^+-$2N_2$), 376, 358, 335, 318, 275, 258 (Found: C, 58·48; H, 6·66; N, 11·53. $C_{23}H_{30}N_4O_7$ requires: C, 58·21; H, 6·37; N, 11·81%).

Tetrahydroerioflorin acetate. Reduction of erioflorin acetate (106 mg) with H_2 over PtO_2 in EtOH afforded the tetrahydro-derivative (97 mg, 91%), m.p. 199–201° (EtOAc–hexane) (lit.[8] 213–218°), $[\alpha]_D$ −73° (*c* 1·0, $CHCl_3$), *m/e* 352 (M^+-ketene), 334, 323, 306, 263 (base peak), 246, 137 (Found: C, 63·74; H, 7·54. Calc. for $C_{21}H_{30}O_7$, C, 63·94; H, 7·66%).

Ovatifolin (X) (R_f 0·25) and its acetate (XI). Repeated crystallization of this fraction from hexane–EtOAc eventually afforded a pure sample of *ovatifolin*, m.p. 131–134°, $[\alpha]_D$ −75° (*c* 1·2, $CHCl_3$), λ_{max} 209 nm (ϵ 16 800), ν_{max} 3454, 1747, 1738, 1658, 1297, 1237, 1169, 1027, 979 cm^{-1}, *m/e* 306 (M^+, very weak), 264, 246, 228, 213, 166, 121, 107, 91, 43, for NMR bands see Table 1 (Found: C, 66·25; H, 7·00, $C_{17}H_{22}O_5$ requires: C, 66·60; H, 7·21%). Acetylation of the crude material afforded both erioflorin acetate (see above) and *ovatifolin acetate* (XI), which was slightly the less polar of the two. The latter had m.p. 152–154° (no visible decomposition but the sample changed, by TLC examination of the sample after melting), $[\alpha]_D$ −13° (*c* 1·2, $CHCl_3$), λ_{max} 212 nm (ϵ 10 000), ν_{max} 1769–1762, 1739, 1733, 1662, 1258, 1242, 1236, 1138, 1054 and 973 cm^{-1}, see Table 1 for NMR, *m/e* 348 (M^+, very weak), 306, 288, 246, 228, 213, 200, 186. This material was identical in all respects to an authentic sample of zexbrevin D.[14]

Reduction of ovatifolin acetate. The acetate (75 mg) in EtOH was reduced with H_2, using PtO_2 as catalyst. One major product was isolated by TLC, which crystallized from Et_2O–hexane as needles of *deacetoxy-hexahydro-ovatifolin acetate* (XII) (28 mg, 44%), m.p. 115–118°, $[\alpha]_D$ −65° (*c* 0·4, EtOH), ν_{max} 1772, 1729, 1280, 1242, 1188, 1125, 1076, 1024, and 990 cm^{-1}; τ 4·86–5·24 (2H, *m*), 7·24 (1H, *bd*, *J*, *ca.* 4 Hz), 7·34 (1H, *m*), 8·08 (3H, *s*), 8·93 (3H, *d*, *J* 7 Hz), 9·05 (6H, *d*, *J* 6·5 Hz), and methylene protons; *m/e* 296 (M^+), 281, 279, 236, 208, 180, 163, 141, 123, 109, 95 and 43 (base peak) (Found: C, 69·08; H, 9·50. $C_{17}H_{28}O_4$ requires: C, 68·89; H, 9·52%).

Reduction of epitulipinolide. Hydrogenation of an authentic sample of epitulipinolide under the same conditions employed for ovatifolin acetate afforded a *hexahydro-derivative*, m.p. 116–117° (isopropyl ether) and 117–120° (Et_2O–hexane), $[\alpha]_D$ −92° (*c* 0·9, $CHCl_3$), ν_{max} 1770, 1731, 1246, 1188, 1024, 988 cm^{-1}; τ 4·86–5·24 (2H, *m*), 7·14–7·35 (2H, *m*), 8·08 (3H, *s*), 8·93 (3H, *d*, *J* 6·5 Hz), 9·05 (6H, two overlapping doublets, *J* 5·0, 6·0 Hz), and methylene protons; *m/e* 296 (281), 279, 236, 208 (similar to that for compound XII) (Found: C, 69·24, H, 9·44. $C_{17}H_{28}O_4$ requires: C, 68·89; H, 9·52%).

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