Phytochemistry, 1969, Vol. 8, pp. 2381 to 2392. Pergamon Press. Printed in England

SESQUITERPENE LACTONES. THE CONSTITUENTS OF *ERIOPHYLLUM CONFERTIFLORUM*

S. J. TORRANCE, T. A. GEISSMAN and M. R. CHEDEKEL

Department of Chemistry, University of California,[1] Los Angeles, California 90024

(*Received* 16 *June* 1969)

Abstract—Six new sesquiterpene lactones, closely allied in structure, have been isolated from *Eriophyllum confertiflorum* Gray, and their structures established. One of them is the methacrylyl ester of a lactone which, as the tiglic acid ester, occurs in *Helianthus tuberosus* L.

INTRODUCTION

Eriophyllum confertiflorum Gray[2] is a common member, abundant in California, of the tribe Helenieae (family Compositae). The plant is rich in a number of sesquiterpene lactones, six of which have been separated and characterized. The structures of erioflorin (I), eriophyllin (II), eriophyllin-B (deacetyleriophyllin) (III), and eriophyllin-C (deacetyldehydroeriophyllin) (IV) have been determined, and provisional structures are put forward for some minor constituents which were obtained in amounts too small for exhaustive study.

RESULTS AND DISCUSSION

Erioflorin (I), $C_{19}H_{24}O_6$, is the methacrylic ester of a sesquiterpene lactone, the structure of which corresponds to that reported[3] for helianginol, the hydrolysis product of the tiglic acid ester heliangine, isolated from *Helianthus tuberosus* L. The structure of erioflorin was established by the studies described in what follows, and is supported by parallel studies on three accompanying constituents of the plant: eriophyllin, eriophyllin-B (deacetyleriophyllin), and eriophyllin-C (deacetyldehydroeriophyllin). In addition to these four lactones, the plant contains at least two others, eriolin and hydroxyeriolin, to which structures have been assigned on the basis of their physical properties and their biosynthetic relationship to erioflorin and its allies.

The nature of the acyl grouping of erioflorin was revealed by the NMR spectrum, which showed signals for the methyl and methylene groups of the methacrylyl residue at δ 1·92 (3H, broad singlet), 5·62 (1H, broad singlet) and 6·12 (1H, broad singlet) which were nearly identical in form and position with those of ethyl methacrylate. Methanolysis of erioflorin yielded methyl methacrylate, identified by its retention time in a gas chromatogram. Tetrahydroacetylerioflorin (V) yielded isobutyric acid upon alkaline hydrolysis. The acid was

[1] Contribution No. 2415 from the Department of Chemistry, UCLA.

[2] Several collections of the plant from Bouquet Canyon, near Saugus, California, identified by Voucher Numbers 41567-ECL, 41766-ECL, 51166-ECL and 51466-EC, and from Tuna Canyon of the Santa Monica Mountains, identified by Voucher Numbers 61967-ECL and 70467-ECL, were substantially identical in composition (by TLC analysis) and are not separately treated here.

[3] H. MORIMOTO, Y. SANNO and H. OSHIO, *Tetrahedron* **22**, 3173 (1966).

identified by its NMR spectrum and by preparation of the anilide, identical with an authentic specimen.

The lactone grouping of erioflorin was identified as the α-methylene-γ-lactone by the characteristic NMR signals for the methylene protons (δ 5·83, 6·35; doublets, $J=2$ Hz), and by the i.r. spectrum (1770 and 1650 cm^{-1}). The presence of a vinylic methyl group was disclosed by the NMR signals at δ 1·84 (3H, d, $J=0{\cdot}5$ Hz) and 5·31 (1H, d, $J=1{\cdot}1$ Hz, broadened by allylic coupling to $-CH_3$). Further evidence for the presence of the grouping $CH_3-\underset{|}{C}=CH-$ in erioflorin is found in the structure of eriophyllin-C (deacetyldehydroeriophyllin) (IV), a compound which differs from erioflorin only in the presence of the grouping $-CH=\underset{|}{C}-CHO$ in place of $-CH=\underset{|}{C}-CH_3$. In the NMR spectrum of IV, the methyl group signal (at δ 1·84 in erioflorin) is absent, and the one-proton signal (at 5·31 in erioflorin) is seen at 6·37 as a doublet of which each component is now sharp. Eriophyllin-C showed the aldehydic proton as a 1-H singlet at δ 9·46.

Erioflorin possesses a secondary hydroxyl group. It shows i.r. absorption at 3570 and 3430 cm^{-1}, and a one-proton signal in the NMR at δ 3·37 (1H, br, s), which is removed when deuterium oxide is added. The proton of the $-CH-OH$ group is seen as a one-proton multiplet at δ 4·50, and this is displaced to δ 5·20 in the spectrum of erioflorin acetate (XV).

The presence of an oxide ring in erioflorin was inferred from its composition and the absence of additional functionality necessary to accommodate the sixth oxygen atom of the formula. An NMR signal at δ 2·37 (1H, t, $J=4$ Hz) indicates that this is an epoxide, and from the position (δ 1·50, 3H, s) of the signal for the methyl group it is concluded that the epoxide is in the position $CH_3-\overset{O}{\overset{/\backslash}{C}}-CH$.

Substantiation of the presence and location of the epoxide grouping was found in the following transformations. Treatment of tetrahydroacetylerioflorin (V) with a trace of mineral acid in acetone resulted in opening the epoxide ring with the formation of the unsaturated alcohol grouping of VI. The NMR spectrum of this compound was in accord with the structure shown. The signal for the methyl group (at δ 1·50 in erioflorin) was absent in the spectrum of VI, and there appeared two one-proton singlets (broad) at δ 5·10 and 5·33 for the protons of the newly formed $=CH_2$ grouping. In addition, a secondary hydroxyl group was present (1H signal at δ 4·0 which moves downfield in the spectrum of the acetate of VI).

Oxidation of VI with manganese dioxide yielded a ketone (VII) whose u.v. (225 nm, $\log\epsilon$ 4·0) and i.r. spectra (1675 cm^{-1}) were in accord with the structure shown. The exocyclic methylene group of the ketone appeared in the NMR spectrum as two one-proton signals at δ 5·80 and 6·03. Reduction of VII yielded the dihydro compound, passage of which through an alumina column resulted in its conversion into the dienone VIII. The i.r. (1690 cm^{-1}) and NMR (absence of the acetyl methyl signal, presence of two additional vinyl proton signals) spectra are in accord with the structure presented for VIII, and its u.v. spectrum showed a maximum at 255 nm. This is not in discord with the structure of VIII because, although the calculated maximum is 281 nm, Dreiding models show that the co-planarity of the dienone system is impossible in the ten-membered ring.

The above information leads to the conclusion that erioflorin is a germacranolide, and that its structure can be represented as I. The transformations described above are formulated

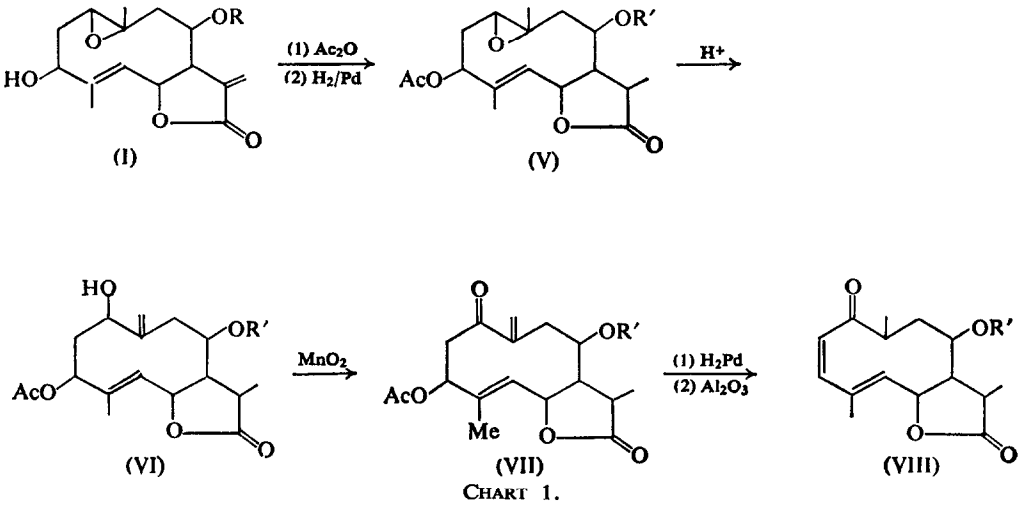


CHART 1.

as in Chart 1 (where R = —CO—$\overset{CH_3}{\overset{|}{C}}$=$CH_2$ and R′ = —$COCH(CH_3)_2$). The relative positions of the functions shown in Chart 1 were established by the following considerations. Of special significance are a pair of one-proton doublets in the spectrum of demethacryldihydroerioflorin (X) (R′ = H) centered at δ 5·52 and 6·50. The former consists of two broadened peaks and is assigned to the vinylic proton at C-5, coupled (with J = 11 Hz) to the proton at C-6 and broadened by allylic coupling with the methyl group at C-4. The signal at δ 6·50 is a pair of doublets with couplings of J = 11 Hz (with H-5) and J = 1 Hz (with H-7). The low-field position of this proton is unusual and suggests that the C-6 proton is β-oriented and is strongly deshielded by the hydroxyl group at C-8, also β-disposed.

The placing of the hydroxyl group of erioflorin at C-3 is established by the observation that chromic acid oxidation (Chart 2) of tetrahydroerioflorin (X) gives an α,β-unsaturated ketone (XI), with an u.v. maximum at 246 nm (log ε 3·4) and an i.r. band at 1685 cm^{-1}. Treatment of erioflorin with 3 moles of hydrogen and 10 per cent palladized charcoal resulted in the hydrogenolysis of this hydroxyl group giving IX, whose i.r. and NMR spectra no longer showed hydroxyl absorption.

Structure X (with R′ = H) is that assigned[3] to dihydrohelianginol. Direct comparison of specimens of X (R′ = H) and dihydrohelianginol[4] was not conclusive, for the specimen of dihydrohelianginol had undergone decomposition when received by us; however, the i.r. and NMR spectra of the two specimens were identical in all respects. Moreover, the NMR spectrum recorded[3] for heliangine corresponds in all respects (except for those of the acyl grouping) to that of erioflorin.

Structure I (with R = H) has been assigned[3] to helianginol. Direct comparison of specimens of I (R = H) and helianginol[5] were also not entirely conclusive, for even though the specimen of helianginol was repeatedly recrystallized it still had a melting point (204–205°) 10° below that of our material (214–218°), but, it will be noted, above that reported.[3]

[4] We thank Dr. Morimoto for a specimen of dihydrohelianginol, and also copies of its NMR and i.r. spectra. The specimen had a reported m.p. of 202–203°, while our X (R′ = H) had m.p. 219–220°.

[5] We also thank Dr. Morimoto for a specimen and copies of spectra of helianginol. The specimen had a reported m.p. of 191–192°, while our material (I, R = H) had m.p. 214–218°.

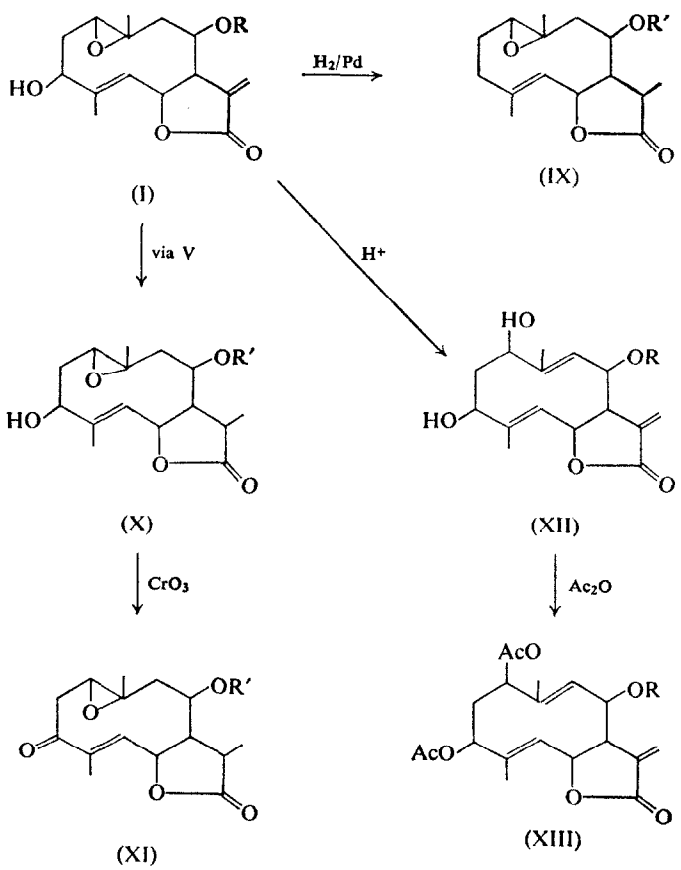
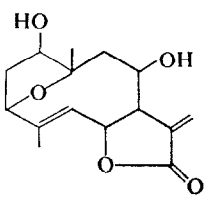


CHART 2.

However, a mixed melting point of our material and helianginol was not depressed, and the i.r. and NMR spectra of the two specimens were identical in all respects.[6]

[6] The poor yields recorded[3] for the hydrolysis of heliangine and its derivatives are paralleled by our experience. Indeed, we have reason to believe that interaction of one or both of the hydroxyl groups with the epoxide occurs during alkaline hydrolysis, giving mixtures from which isolation of pure components is difficult. For example, hydrolysis of erioflorin results in the rearrangement product *a* (the structure of which is not described here)[7] in greater yield than the unrearranged product I (R = H).



(*a*)

[7] S. J. TORRANCE, M.S. Thesis, University of California, Los Angeles (1969).

The location of the methacrylate grouping at C-8 rather than C-9 is suggested by the fact that no known germacranolide contains oxygenation at C-9. Experimental evidence in verification of this is that acid treatment of erioflorin (Chart 2) yields a compound formulated as XII. The NMR spectrum of XII shows three vinyl methyl groups (6H, s, δ 1·82; 3H, s, 1·95), and two additional downfield protons which were not present in the spectrum of erioflorin. One of these is the proton at C-9; the other is the proton at the hydroxyl-bearing atom C-1 (1H, 4·60, which moves downfield to 5·40 in the acetate (XIII) of XII).

Further evidence for the structure of erioflorin is found in the study of the closely related eriophyllin (II) which occurs as the second major constituent of *Eriophyllum confertiflorum*. Eriophyllin, $C_{21}H_{26}O_8$, contains a methacryl grouping, an acetyl group, and an epoxide; it is an α-methylene-γ-lactone. These features are readily recognized in the NMR spectrum, in which they are seen as signals resembling those described for erioflorin. The most conspicuous difference between the NMR spectra of erioflorin and eriophyllin is the absence in the latter of the signal for the C-4 vinyl methyl group and, in its stead, the presence of a two-proton singlet (broad) at δ 4·25, which moves to δ 4·67 upon acetylation. That this signal represents the $HOCH_2—\overset{|}{C}=\overset{|}{C}H$ grouping was shown by the oxidation of eriophyllin with manganese dioxide. The product (XIV) was an aldehyde; the signal for the methylene grouping of $—CH_2OH$ was absent from the NMR spectrum, and there appeared a one-proton singlet at δ 9·46 for the proton of the —CHO group. A direct relationship between erioflorin and eriophyllin was established by hydrogenation of acetylerioflorin (XV) and eriophyllin, with the formation of acetyltetrahydroerioflorin (V) from both. The hydrogenolysis of the $—CH_2OH$ group of eriophyllin (as well as its oxidation by manganese dioxide) establishes the allylic nature of the $HOCH_2$ grouping in eriophyllin, and the formation of the common product by hydrogenation of eriophyllin and acetylerioflorin shows that these two compounds are related as shown in Chart 3.

Two other compounds present in *E. confertiflorum* are eriophyllin-B (deacetyleriophyllin) (III), and eriophyllin-C (deacetyldehydroeriophyllin) (IV). Of these, the former was readily characterized by its acetylation, with the formation of acetyleriophyllin. Eriophyllin-C

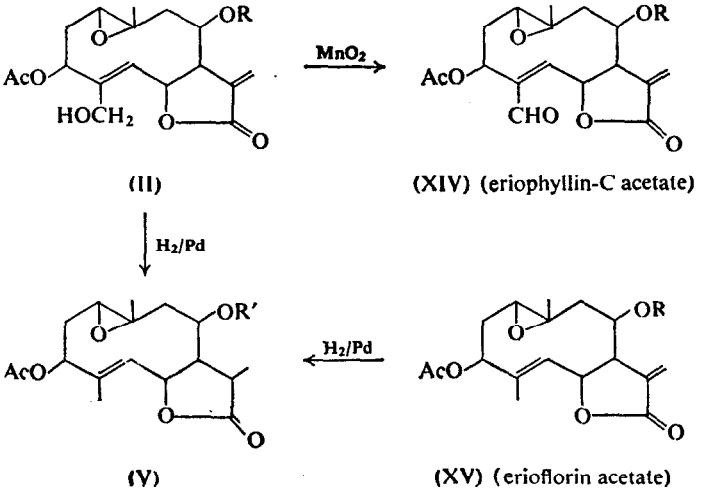
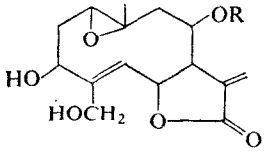
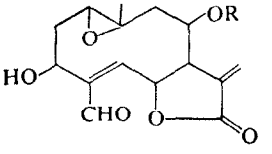


CHART 3.

(deacetyldehydroeriophyllin) is the aldehyde (—CHO at C-4) corresponding to deacetyleriophyllin (III) (—CH_2OH at C-4). Manganese dioxide oxidation of eriophyllin-B (III) readily gave eriophyllin-C (IV).

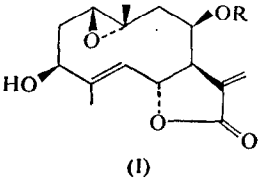


eriophyllin-B (III)
(deacetyleriophyllin)



eriophyllin-C (IV)
(deacetyldehydroeriophyllin)

The stereochemistry of erioflorin and its allies is the following (I), by relation to the X-ray



(I)

crystallography done on heliangine[3] and chemical and physical evidence presented above.

Minor constituents of *E. confertiflorum* are eriolin (XVII) and hydroxyeriolin (XVI). Although these were isolated in amounts too small to permit extensive studies, the structures shown are assigned to them on the basis of their composition and interpretations of their spectral properties, and the assumption that they are closely related in biosynthesis and structure to the compounds that they accompany in the plant.

Hydroxyeriolin (XVI), $C_{15}H_{22}O_5$, shows the typical i.r. and NMR signals for an α-methyl γ-lactone grouping.[8] The u.v. spectrum shows end absorption only (<200 nm), and the NMR spectrum (DMSO-d_6) lacks the characteristic pair of doublets of the α-methylene-γ-lactone grouping. Instead, it contains a three-proton doublet at δ 1·20 (J=7 Hz) for the α-methyl group of the lactone. Indeed, no proton signals are seen below δ 5·0. The presence of a secondary hydroxyl group is shown by a signal at δ 4·90 (d) which disappears when deuterium oxide is added, and by a multiplet signal (1H) at δ 3·60 (in DMSO-d_6) which moves to δ 4·90 (in $CDCl_3$) in hydroxyeriolin acetate.

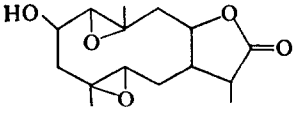
The additional two oxygen atoms are present in ether linkages for, besides the lactone and hydroxyl group, no other ketone, hydroxyl or ester groupings are present. The chemical shifts and multiplicities (δ 1·23 and 1·41, both singlets) of the signals of the two remaining methyl groups, and the lack of —C*H*—O— signals downfield at δ 3·0 (except for the lactone and hydroxyl —C*H*—O—) indicate that two CH_3—C(O)—CH groupings are present. Since, among the known germacranolides, double bonds are present only at the C-1/C-10 and C-4/C-5 bonds, and since epoxides presumably arise biogenetically by direct oxidation of double bonds, these epoxides are assumed to be so situated.

The lactonic proton (—C*H*—O) of hydroxyeriolin is seen as a complex triplet, indicating that it is coupled to more than two adjacent protons. This suggests, bearing in mind the

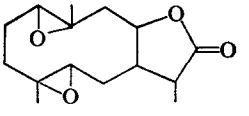
[8] The co-occurrence of α-methylene-γ-lactones and their 11,13-dihydro counterparts is known in several other plants in which this class of compounds is found.

positions of the epoxides, that the lactone is closed at C-8 and not at C-6. If the lactone were closed at C-6, the lactonic proton would be expected to be a clean triplet or quartet, coupled only to H-5 and H-7. The multiplicity of the signal for the proton of —C*H*—OAc in the NMR spectrum of hydroxyeriolin acetate is a clear octet which shows coupling ($J = 5$, 10 and 12 Hz) to three adjacent protons. The only position of attachment in the structure of hydroxyeriolin which would meet this requirement is C-2. The summary of these conclusions is expressed in the proposed structure XVI, in which the stereochemistry is not known.[9]

Eriolin, $C_{15}H_{22}O_4$, was isolated in small amount. Its NMR spectrum shows signals for three methyl groups (δ 1·27, d, $J = 7$ Hz; 1·29, s; 1·40, s); a 1-H complex triplet at δ 4·20 (lactonic proton at C-8); and no other signals at fields lower than δ 3·0. The simplicity of the composition and spectrum of eriolin suggests that it is deoxy-hydroxyeriolin, for the character of the signals for the lactonic (—C*H*—O—) protons in the two compounds, indicating that the lactones are closed at C-8, are nearly identical. The structure is represented as XVII, but this must be regarded as provisional in the absence of further information.



hydroxyeriolin (XVI)



eriolin (XVII)

Recent examination of a new population of *E. confertiflorum*[10] afforded the surprising result that the new collection (from a different location than that in which the two earlier collections were made) bears little or no resemblance in its chemical composition to the material used for the study described in this paper. The new material was devoid of erioflorin and eriophyllin but contained as the principal constituent (in yields as high as 2% of dry plant!) a new compound, called eriofertin. Studies on eriofertin (which does, however, appear to be structurally related to erioflorin, etc.) are now in progress. The implication of this observation in respect to the taxonomy of *E. confertiflorum* is under study.

EXPERIMENTAL

M.ps were determined on a Büchi melting point apparatus and are corrected. U.v. spectra were measured on a Carey recording spectrophotometer, Model 14; i.r. spectra were measured in $CHCl_3$, unless otherwise specified, on Perkin-Elmer Models 237 and 127, Infracord. The listings of i.r. bands include those which are relevant to the structural argument. The NMR spectra were taken in $CDCl_3$, unless otherwise specified, recorded on Varian Associates A-60-A, A-60-D, T-60 and HA-100 instruments, and peak positions are given in δ values with tetramethylsilane as an internal standard. Description of those features of the NMR spectra relevant to the structural arguments are to be found in the Discussion. Mass spectra were obtained on an AIE MS-9 mass spectrometer. Thin-layer chromatograms were prepared with Baker silica gel G; also used were Merck F_{254} precoated 5 × 10 cm silica gel slides. In every case the solvent system used was acetone–$CHCl_3$ (2:3); the plates were developed by spraying with conc. H_2SO_4 and then heating. Baker silica gel and Woelm neutral alumina were used for column chromatography.

Extraction of the Plant Material

The *Eriophyllum confertiflorum*[2] was oven-dried at 60° overnight, ground in a Wiley mill and extracted with $CHCl_3$ by either soxhlet or percolation techniques. The workup of all of the plant collections was

[9] It may be noted that the co-occurrence in a single plant of closely related compounds differing in that one has OH at C-2 and the other OH at C-3 is not without precedent; N. H. FISCHER and T. J. MABRY, *Chem. Commun.* 1235 (1967); N. H. FISCHER, T. J. MABRY and H. B. KAGAN, *Tetrahedron* **24**, 3091 (1968).

[10] Voucher specimens of the collections (*a*) from which erioflorin and allies were isolated, and (*b*) the recent collection, have been examined by Professor Lincoln Constance. He reported that, in his opinion, both would be identified as the single taxon, *E. confertiflorum* Gray.

substantially alike, and therefore the total workup of collection 70467-ECL is described in detail and used as an example for all the other plant collections.

6930 g of dried and ground 70467-ECL was placed in a large soxhlet (receiver flask capacity, 50 l.) and extracted with 60 l. $CHCl_3$ for 5 days. The $CHCl_3$ extract was evaporated in a Cyclone evaporator and the resultant thick green-black tar dissolved in 1 l. ethanol–water (1:3) and boiled with 10 g of Norite for 30 min. The hot solution was filtered through a Celite pad in a steamjacketed Büchner funnel and the resulting yellow filtrate extracted with $CHCl_3$ (8 l.) until the extract no longer showed sesquiterpenic constituents (by TLC). The $CHCl_3$ was removed *in vacuo* to yield a thick yellow-brown oil (550 g).

Chromatography

From the above oil crystallized 10·6 g of eriophyllin which was collected by triturating the oil with ethyl ether, filtering and washing the crystals with more ether. The ether was then removed *in vacuo* from the filtrate, the residue taken up in 200 ml of benzene–$CHCl_3$ (1:1), and chromatographed over silica gel (12 × 150 cm). The column was eluted with 2 l. benzene–$CHCl_3$ (1:1), then 45 l. $CHCl_3$, and finally 25 l. of acetone–$CHCl_3$ (1:5). Fractions of 125 ml were collected; the first nine contained nothing, the second twenty-five yielded waxes and plant pigments, and fractions 34–413 gave a total of 14·2 g of erioflorin. Fractions 414–620 were found by TLC to contain several compounds but the major constituent was eriophyllin. These fractions were combined and taken up in ethanol, from which 28·6 g of eriophyllin was crystallized.

Erioflorin (I)

The 14·2 g of erioflorin obtained from the above column eluates crystallized from ethyl acetate as colorless prisms, m.p. 236–238° dec., $[\alpha]_D^{25}$ −100° (*c.* 1·0, $CHCl_3$); u.v. 210 nm, log ε 4·31; i.r. 3570, 3420, 1770, 1725, 1650 cm^{-1}. (Found: C, 65·67; 65·72; H, 7·25, 7·26. Calc. for $C_{19}H_{24}O_6$: C, 65·50; H, 6·94. Calc. for $C_{20}H_{26}O_6$: C. 66·28; H, 7·23%.) Mass spectrum: *m/e* 348 and 362.[11]

Eriophyllin (II)

The product (39·2 g) obtained by crystallization of the plant extract and from the column eluates described above was recrystallized from ethanol, forming colorless needles, m.p. 220–222° dec. It had $[\alpha]_D^{25}$ −118° (*c.* 1·0, $CHCl_3$); u.v. 210 nm, log ε 4·62; i.r. 3500, 1770, 1750, 1720, 1640 cm^{-1}. (Found: C, 62·73, 62·78; H, 6·76, 6·62. Calc. for $C_{21}H_{26}O_8$: C, 62·06; H, 6·45. Calc. for $C_{22}H_{28}O_8$: C, 62·84; H, 6·71. Mass spectrum: *m/e* 406 and 420.[11]

Eriophyllin-B (III) and Eriophyllin-C (IV)

The oil (about 60 g) of column fractions 414–620 from which eriophyllin was crystallized was rechromatographed over silica ge (7 × 50 cm), eluting with a $CHCl_3$–methanol mixture, gradually increasing the concentration of the latter (up to 10 per cent). From the $CHCl_3$ eluates was isolated 50 mg of eriophyllin-C (IV) as a white powder, m.p. 166–169° (dec.); i.r. bands at 3600–3300, 1770, 1720, 1690, and 1640 cm^{-1}, u.v. 210 nm (log ε 4·50); $[\alpha]_{5460}^{25}$ −65·8°; molecular weight (mass spectrum) 362 and 376. We have noted above[11] that eriophyllin-C ($C_{19}H_{22}O_7$) was found to be contaminated with $C_{20}H_{24}O_7$. Attempts at recrystallization resulted in the decomposition of the very unstable eriophyllin-C.

From the eluates corresponding to methanol–$CHCl_3$ (1:10) was isolated 3·5 g of eriophyllin-B (III) as a yellow oil which could not be induced to crystallize; $[\alpha]_{5460}^{25}$ −72·6°. However, the NMR spectrum of eriophyllin-B was nearly identical to that of erioflorin except that in place of the three-proton CH_3—C=CH signal at δ 1·84 in the latter there appeared a two-proton $HOCH_2$—C=CH signal at δ 4·25 in the former. This indicated that eriophyllin-B was deacetyleriophyllin. Experiments that demonstrate the structures of eriophyllin-B and -C are described in the following.

Eriolin (XVII)

The mother liquor of column fractions 34–413 from which erioflorin was crystallized yielded 100 mg of eriolin after trituration with ether and crystallization by concentration and cooling of the solution to 0°. Eriolin was found to behave chromatographically identically to erioflorin and it was concluded that the

[11] Each of the four principal constituents (I–IV) was accompanied by a persistent contaminant, from which it could not be completely freed by chromatography or recrystallization, differing from the principal compound by the unit CH_2, the increment being present in a homologous acyl grouping. The mass spectra of the compounds contained, in addition to the principal molecular ion M, a second peak at M + 14. Thus, although there is some uncertainty regarding the properties of the pure compounds I–IV, their derivatives and transformation products in nearly all cases lost the contaminant in the process of manipulation and purification, and served as the basis for the structural conclusions described in the Discussion. Eriolin and hydroxyeriolin (which contain no acyl groupings) were isolated in the uncontaminated state.

erioflorin from 70467-ECL was probably contaminated with eriolin. Fractional recrystallization of this erioflorin resulted in a few more mg of eriolin, but all of it could not be separated. Eriolin was obtained in the same fashion from 61967-ECL but not from any of the other plant collections. Eriolin had m.p. 238–240° (dec.); $[\alpha]_D^{24}$ −42° (*c.*, 1·0 $CHCl_3$); the only significant i.r. band was 1776 cm^{-1}. (Found: C, 67·69; H, 8·40. Calc. for $C_{15}H_{22}O_4$: C, 67·64; H, 8·33%.) Molecular weight (mass spectrum) 266.

Hydroxyeriolin (XVI)

Hydroxyeriolin was found to be chromatographically inseparable from eriophyllin (column and TLC) and only a few mg of hydroxyeriolin could be obtained by fractional recrystallization of the eriophyllin from 70467-ECL. However, even though hydroxyeriolin co-crystallized with eriophyllin, the former was found to be much more inert than the latter. Therefore, degradations of eriophyllin usually resulted in unreacted hydroxyeriolin which could then be isolated by chromatography. For example, MnO_2 oxidation of 1·19 g of eriophyllin (II) permitted the isolation of 0·16 g of hydroxyeriolin; and hydrogenation of 36·9 g of eriophyllin resulted in the isolation of 4·63 g of hydroxyeriolin. Hydroxyeriolin was obtained in the same fashion from eriophyllin from 61967-ECL but not from any of the other plant collections. Hydroxyeriolin had m.p. 256–260° (dec.); $[\alpha]_D^{25}$ −13° (*c.*, 1·0 pyridine); and i.r. (KBr) bands at 3500 and 1760 cm^{-1}. (Found: C, 63·84; H, 7·94. Calc. for $C_{15}H_{22}O_5$: C, 63·81; H, 7·85%.) Molecular weight (mass spectrum) 282.

Methyl Methacrylate from Erioflorin (I)

To 1·01 g of erioflorin was added 0·60 g (1·5 equivalents) of K_2CO_3 dissolved in 7 ml of water and 15 ml of methanol. The mixture was stirred to effect dissolution of the erioflorin, stoppered, and allowed to stand under N_2 at room temp. for 4·5 hr (after which time the characteristic odor of methyl methacrylate was detected). The reaction mixture was diluted with 250 ml of water and extracted with ether (2 × 50 ml). The ethereal extracts were combined, dried, and evaporated to a volume of 5 ml. This solution of methyl methacrylate was found to be identical in retention time to an authentic sample (also in ether solution) in gas chromatograms over both Carbowax and alumina columns.

Tetrahydroacetylerioflorin (V) from Erioflorin (I)

A solution of 0·95 g of erioflorin in 3 ml of pyridine and 3 ml Ac_2O was allowed to stand at room temp. overnight. The solvents were then removed *in vacuo* and the crystalline residue (shown by TLC to be erioflorin acetate (XV)) was dissolved in 50 ml EtOAc. To this was added 150 mg of palladized charcoal and the mixture was hydrogenated at room temp. and atmospheric pressure for 6 hr. 2 moles of hydrogen were absorbed. The catalyst was removed by filtration through Celite and the solvent removed *in vacuo*. The resulting crystalline residue was recrystallized from 95% ethanol to give 0·63 g of tetrahydroacetylerioflorin (V) as large colorless needles, m.p. 213–218° (dec.); i.r. bands at 1770 and 1730 cm^{-1}. (Found: C, 64·20; H, 7·72. Calc. for $C_{21}H_{30}O_7$: C, 63·94; H, 7·66. Calc. for $C_{22}H_{32}O_7$: C, 64·68; H, 7·90%.) Molecular weight (mass spectrum) 394 and 408.

Isobutyric Acid from Tetrahydroacetylerioflorin (V)

To 8·60 of V dissolved in 100 ml of methanol was added 4·3 g KOH (approx. three equivalents) dissolved in 50 ml of water. This solution was refluxed on a steam bath for 50 min, acidified with conc. HCl, and the methanol removed *in vacuo* to a final volume of 100 ml. This aqueous solution was extracted with $CHCl_3$ (5 × 100 ml) which were then combined, concentrated to a volume of 250 ml, and extracted with sat. $NaHCO_3$ (2 × 100 ml). The bicarbonate extracts were combined, acidified, and extracted with $CHCl_3$ (2 × 150 ml) which were combined and dried. TLC showed that this extract still contained non-acidic material; the solvent was therefore removed and the residue dissolved in ether and the solution extracted with 100 ml of sat. $NaHCO_3$, which was then acidified and extracted with 100 ml of ether. Removal of the solvent afforded 1·06 g of isobutyric acid, b.p. 156°. The NMR spectra (neat) of this and an authentic sample of isobutyric acid were identical in all respects.

Anilide of the Isobutyric Acid from V

From 0·62 g of isobutyric acid (from the hydrolysis of V) was prepared 26 mg of the anilide, by the method of Kuehn and McElvain,[12] as colorless needles, m.p. 103–104°.

The anilide of an authentic sample of isobutyric acid prepared as above had m.p. 106–106·5°, and a mixture of the two samples had m.p. 103–105°.

Acetylation of Erioflorin (I)

Acetylation of 1·07 g of erioflorin with pyridine–Ac_2O gave 0·70 g of acetylerioflorin (XV), colorless prisms from ethanol, m.p. 210–212° (dec.). (Found: C, 64·77; H, 6·81. Calc. for $C_{21}H_{26}O_7$: C, 64·62; H, 6·67. Calc. for $C_{22}H_{28}O_7$: C, 65·34; H, 6·93%.) Molecular weight (mass spectrum) 390 and 404.

[12] J. KUEHN and M. A. MCELVAIN, *J. Am. Chem. Soc.* 53, 1173 (1931).

Epoxide-Opening of Tetrahydroacetylerioflorin: Conversion of V to VI

To a solution 5·01 g of tetrahydroacetylerioflorin (V) in 400 ml of acetone was added 12 ml conc. HCl and the mixture refluxed on a steam bath, monitoring the progress of the reaction by TLC. After 5 hr, TLC showed the reaction to have proceeded to form mainly one product. The acetone was removed *in vacuo* and the residue dissolved in 500 ml $CHCl_3$ and extracted with 300 ml of sat. $NaHCO_3$. The $CHCl_3$ layer was washed with 200 ml of water, dried, and the solvent was removed *in vacuo*. The residue eventually crystallized; the crystals were collected and washed with ether, resulting in a yield of 3·05 g of VI as colorless plates, m.p. 198·5–199°; i.r. bands at 3600–3300, 1775, and 1740 cm^{-1}. (Found: C, 64·19, 64·00; H, 7·63, 7·92. Calc. for $C_{21}H_{30}O_7$: C, 63·94; H, 7·66%.) Molecular weight (mass spectrum) 394.

Acetylation of VI

Acetylation of 0·26 g of VI with pyridine–Ac_2O yielded 0·24 g of the acetate of VI as colorless spars and rods (from ether), m.p. 174–175°; i.r. bands at 1775 and 1750 cm^{-1}. (Found: C, 63·41; H, 7·35. Calc. for $C_{23}H_{32}O_8$: C, 63·28; H, 7·39%.) Molecular weight (mass spectrum) 436.

Oxidation of VI to VII

To a solution of 2·55 g of VI in 150 ml CH_2Cl_2 was added 30·6 g (approx. thirty equivalents) of active MnO_2.[13] The reaction mixture was stirred at room temp. under a $CaCl_2$ drying tube for 50 hr. The MnO_2 was removed by filtration through Celite and washed with 300 ml $CHCl_3$. The solvent was removed *in vacuo* and the residue crystallized from ether to give a total of 1·83 g of VII as colorless prisms, m.p. 166–167°; i.r. bands at 1775, 1740, and 1672 cm^{-1}; u.v. maximum at 225 nm ($\log \epsilon$ 4·0). (Found: C, 64·39; H, 7·12. Calc. for $C_{21}H_{28}O_7$: C, 64·27; H, 7·19%.) Molecular weight (mass spectrum) 392.

Conversion of VII to VIII

A sample of 0·99 g of VII was dissolved in 50 ml EtOAc and hydrogenated over 0·23 g Pd-C at room temp. and atmospheric pressure for 90 min, at which time approximately 1 mole of hydrogen was taken up and TLC showed the presence of a single product. The catalyst was removed by filtration through Celite and the solvent evaporated *in vacuo* to yield a colorless oil, which was chromatographed over silica gel (3 × 40 cm) ($CHCl_3$) to give 0·42 g of a similar oil which failed to crystallize. This material, homogeneous on TLC, was presumed to be dihydro-VII and its NMR spectrum confirmed this, for it contained only one three-proton vinyl methyl signal.

All of the non-crystalline dihydro-VII was dissolved in a small amount of benzene and chromatographed over activated alumina (Woelm, activity II, 3 × 40 cm). The first 250 ml of eluate (benzene) afforded 0·16 g of VIII which recrystallized from ethanol as colorless needles, m.p. 123–126°; i.r. bands at 1775, 1730, and 1690 cm^{-1}; u.v. maxima at 255 nm ($\log \epsilon$ 4·0) and 200 nm ($\log \epsilon$ 4·3).

Anal. A high resolution mass spectrum gave a molecular weight for VIII of 334·178 ± 0·001. The calculated value for $C_{19}H_{26}O_5$ is 334·178012.

Elimination of the elements of acetic acid from VII (without reduction) proceeded smoothly, but the product was very unstable; hence the use of dihydro-VII for this reaction.

Hydrogenolysis of Erioflorin (I) to (IX)

A solution of 3·43 g of erioflorin in 175 ml HOAc was hydrogenated over 0·68 g of 10% Pd-C at room temp. and atmospheric pressure for 21 hr. The catalyst was removed by filtration through Celite and the solvent evaporated *in vacuo* to yield a colorless oil, which was chromatographed over silica gel (6 × 56 cm). The first 300 ml of eluate ($CHCl_3$) were concentrated and rechromatographed over silica gel (3 × 40 cm) (1:1 benzene–chloroform). The first 200 ml of eluate yielded crystals, which were collected, washed with ether, and recrystallized from ethanol to give 0·37 g of IX as colorless needles, m.p. 165–165·5°; i.r. bands at 1770 and 1735 cm^{-1}. (Found: C, 67·70; H, 8·48. Calc. for $C_{19}H_{28}O_5$: C, 67·83; H, 8·39%.) Molecular weight (mass spectrum) 336.

Tetrahydroerioflorin (X) and Demethyacryldihydroerioflorin (X, R′ = H)

To a solution of 4·04 g of tetrahydroacetylerioflorin (V) in 50 ml of methanol was added 2·0 g (approximately three equivalents) KOH in 40 ml of water. The solution was refluxed for 10 min, diluted with 40 ml of water and extracted continuously with ether for 15 hr. The ether extract was drawn off, dried and set aside. The aqueous layer was acidified with conc. HCl and extracted continuously with ether for 48 hr. The ether extract was drawn off, extracted with sat. $KHCO_3$, dried and set aside. The bicarbonate layer was acidified and extracted with 250 ml ether, from which was isolated about 0·5 g of isobutyric acid. The acidified bicarbonate solution was found by TLC to still contain non-acidic products (demethacryldihydroerioflorin is quite water-soluble) and was therefore continuously extracted with ether for 67 hr. All of the above non-acidic ether extracts were found to be homogeneous by TLC; they were therefore combined and chromatographed

[13] Prepared according to S. BALL, T. W. GOODWIN and R. A. MORTON, *Biochem. J.* **42**, 516 (1949).

over silica gel (3 × 40 cm). $CHCl_3$ elution resulted in the isolation of 0·20 g of tetrahydroerioflorin (X) as colorless needles, m.p. 207–210°; i.r. bands at 3600–3300, 1770 and 1740 cm^{-1}. (Found: C, 64·84; H, 7·69. Calc. for $C_{19}H_{28}O_6$: C, 64·77; H, 7·68. Calc. for $C_{20}H_{28}O_6$: C, 65·93; H, 7·70%.) Molecular weight (mass spectrum) 352 and 364.

Continuation of the chromatography with acetone–$CHCl_3$ (1:5) furnished 4·02 g of demethacryldihydroerioflorin (X, R′ = H), which crystallized from EtOAc as colorless needles, m.p. 219–220° (dec.); i.r. (KBr) bands at 3500, 3480, 1750 and 1670 cm^{-1}. (Found: C, 63·82; H, 7·91. Calc. for $C_{15}H_{22}O_5$: C, 63·81; H, 7·85%.) Molecular weight (mass spectrum) 282.

Oxidation of Tetrahydroerioflorin (X) to (XI)

To 0·06 g of tetrahydroerioflorin dissolved in 5 ml of acetone was added a chromic acid solution (Jones reagent[14]) until the orange-red color persisted (0·43 ml). The solution was diluted with 100 ml of water and extracted with $CHCl_3$ (2 × 125 ml). Removal of the chloroform *in vacuo* afforded a crystalline residue. Recrystallized from ether, this gave a total of 0·04 g of XI as colorless needles, m.p. 165–167°; i.r. bands at 1776, 1730, and 1670 cm^{-1}; u.v. maximum at 246 nm ($\log\epsilon$ 3·7). (Found: C, 65·43; H, 7·45. Calc. for $C_{19}H_{26}O_6$: C, 65·12; H, 7·48%.) Molecular weight (mass spectrum) 350.

Demethacrylerioflorin (I, R = H)

To 1·00 g of erioflorin dissolved in 25 ml of ethanol was added 0·33 g (approx. two equivalents) NaOH in 10 ml of water. The mixture was heated on a steam bath for 5 min, cooled, diluted with 100 ml of water, and worked up in the same manner as in the hydrolysis of V (above). The ether extracts were combined and evaporation of the solvent *in vacuo* resulted in the crystallization of demethacrylerioflorin (I, R = H) as colorless needles, m.p. 191–193° (dec.). Two recrystallizations from EtOAc gave a total of 0·072 g of demethacrylerioflorin as colorless platelets, m.p. 214–218° (dec.); i.r. (KBr) bands at 3500, 3470, 1750 and 1670 cm^{-1}. (Found: C, 64·35; H, 7·19. Calc. for $C_{15}H_{20}O_5$: C, 64·27; H, 7·19%.) Molecular weight (mass spectrum) 280.

Epoxide-Opening of Erioflorin (I) to (XII)

A solution of 1·46 g of erioflorin in a mixture of 100 ml of acetone and 2 ml of conc. HCl was refluxed for 2 hr and worked up as in the case of epoxide opening of V (above). The residue obtained by the evaporation of the solvent was chromatographed over silica gel (3 × 40 cm), eluting with $CHCl_3$ and collecting one hundred 25-ml fractions. Fractions 13–25 yielded 0·37 g of XII as white-brown powdery needles, m.p. 185–195° (dec.); i.r. bands at 3600–3300, 1770, 1720, 1670 and 1650 cm^{-1}. The product was sensitive to manipulation and so was not completely characterized, but was acetylated to yield XIII.

Acetylation of XII to XIII

Acetylation of 0·11 g of XII in pyridine–Ac_2O to give XIII (diacetyl-XII) as an oil which could not be induced to crystallize. This material, however, showed the absence of hydroxyl absorption in its i.r. spectrum, and its NMR spectrum contained two three-proton CH_3COO— signals clearly showing that XII contains two secondary hydroxyl groups.

Acetylation of Eriophyllin (II)

Acetylation of 0·47 g of eriophyllin (II) with pyridine–Ac_2O gave eriophyllin acetate, which crystallized from ethyl acetate as colorless needles, m.p. 171–172°; i.r. bands at 1770, 1750 and 1720 cm^{-1}. (Found: C, 61·98; H, 6·46; C, 62·00; H, 6·45. Calc. for $C_{23}H_{28}O_9$: C, 61·60; H, 6·29. Calc. for $C_{24}H_{30}O_9$: C, 62·32; H, 6·54%.) Molecular weight (mass spectrum) 448 and 462.

Oxidation of Eriophyllin (II) to XIV

Oxidation of 1·19 g of eriophyllin with active MnO_2 yielded a colorless oil. TLC showed that this oil was a mixture of two compounds; therefore, it was chromatographed over silica gel (3 × 40 cm), eluting with $CHCl_3$ and collecting fifty 25-ml fractions. Fractions 27–50 yielded the lower R_f compound (the same R_f on TLC as eriophyllin). This was found to be hydroxyeriolin (see above), present as a constituent of the sample of eriophyllin used. The yield of hydroxyeriolin was 0·16 g.

Fractions 2–22 yielded the higher R_f material which was found to be XIV (eriophyllin-C acetate). The compound (XIV) was not crystalline and polymerized upon attempted crystallization. This oil did, however, show an i.r. band at 1690 cm^{-1} and its NMR spectrum showed a one-proton signal at δ 9·46 indicating that XIV is the expected α,β-unsaturated aldehyde.

Tetrahydroacetylerioflorin (V) from Eriophyllin (II)

A solution of 36·9 g of eriophyllin (II) in 350 ml HOAc was hydrogenated over 2·33 g of 10% Pd-C at room temp. and atmospheric pressure for 48 hr. 3 moles of hydrogen were absorbed. The catalyst was

[14] Prepared according to A. BOWERS, T. G. HALSALL, E. R. H. JONES and A. J. LENIN, *J. Chem. Soc.* 2548 (1953).

removed by filtration through Celite and the solvent was removed *in vacuo* to yield a crystalline residue. Attempts at fractional crystallization of the several components of this residue failed, and the residue was chromatographed over silica gel (5 × 50 cm), collecting three hundred 25-ml fractions and eluting with a $CHCl_3$–acetone mixture, gradually increasing the concentration of the latter. Fractions 176–300 yielded 4·63 g of hydroxyeriolin which had cocrystallized from the plant extract with eriophyllin, along with a non-hydrogenolyzed product (not characterized). Fractions 21–175 yielded tetrahydroacetylerioflorin (V) which, when recrystallized from ethanol, gave a total of 25·5 g of large needles, m.p. 217–220° (dec.); i.r. bands, 1777 and 1730 cm^{-1}. This material was identical with tetrahydroacetylerioflorin obtained from erioflorin, as shown by an undepressed mixed m.p. and the complete identity of the i.r. and NMR spectra of the two samples.

Acetylation of Eriophyllin-B (III) to Acetyleriophyllin

Acetylation of 0·53 g of eriophyllin-B with pyridine–Ac_2O gave 0·40 g acetyleriophyllin as colorless needles (from ethyl acetate), m.p. 173–174°. This material was identical with the acetate of eriophyllin, as shown by an undepressed mixture m.p. and the complete identity of their i.r. and NMR spectra.

Oxidation of Eriophyllin-B (III) to Eriophyllin-C (IV)

Oxidation of 0·83 g of eriophyllin-B with active MnO_2 yielded 0·26 g of eriophyllin-C as a white powder, m.p. 174–176° (dec.). This material was shown to be identical to the naturally occurring eriophyllin-C by an undepressed mixed m.p. and by the identical i.r. spectra of the two samples.

Acetylation of Hydroxyeriolin (XVI)

Acetylation of 0·15 g of hydroxyeriolin in pyridine–Ac_2O gave 0·12 g of hydroxyeriolin acetate as colorless platelets (from $CHCl_3$), m.p. 196–196·2°. The i.r. spectrum of hydroxyeriolin acetate was similar to that of hydroxyeriolin except that it showed no hydroxyl absorption and contained an acetate carbonyl band at 1735 cm^{-1}. (Found: C, 63·17; H, 7·42. Calc. for $C_{17}H_{24}O_6$: C, 62·95; H, 7·46%.) Molecular weight (mass spectrum) 324.

Acknowledgements—This work was carried out with the aid of a grant, GM-14240-03, from the U.S. Public Health Service. The NMR and mass spectrometers were provided in part by funds granted to the Chemistry Department by the National Science Foundation and by E. I. du Pont de Nemours and Company. Mr. T. S. Griffin assisted in some of the early parts of the study. Analyses are by Miss Heather King, UCLA.