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SESQUITERPENE LACTONES OF *ARTEMISIA*— *A. VERLOTORUM* AND *A. VULGARIS**

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Abstract—Three new sesquiterpene lactones have been isolated from a European *Artemisia verlotorum* Lamotte. They show a close structural relationship to other lactones from allied spp. of the *vulgaris* complex and provide further insight into probable biosynthetic pathways in the genus.

INTRODUCTION

EXAMINATION of a number of species of *Artemisia* belonging to the subsection of *Arbrotanum* described as the *vulgaris* complex[1] has shown that the chemistry of the members of this group, based upon their sesquiterpene lactones, reflects the close alliance between them.[2-6] It was therefore of interest to examine two additional species of the class that became available: *Artemisia verlotorum* Lamotte and *A. vulgaris* L.

The study of these plants was of special importance with respect to earlier studies[2] on a plant, collected in Australia, which had been identified by separate authorities as *A. verlotorum* Lamotte and *A. vulgaris* L. The *A. verlotorum* used in this study† is abundant in south central Europe,[7] although its habitat is presumably western China. *A. vulgaris* L.‡ is widely distributed in Europe, common in waste places in urban areas. It is clearly distinguishable from *A. verlotorum* in morphology, habit, and time of flowering.

RESULTS AND DISCUSSION

The results of examining *Artemisia vulgaris* and the European *A. verlotorum* were, in brief, that neither resembled the earlier, Australian, specimen, whose principal sesquiterpene constituent, vulgarin (I), was lacking in both; and neither bore any chemical resemblance to the other.

A. vulgaris was found to be singularly devoid of isolable constituents. It contained only a small amount of extractable material from which no crystallizable or characterizable compounds could be isolated. Vulgarin could not be detected by chromatographic methods.

A. verlotorum, on the other hand, yielded three crystalline sesquiterpenoid lactones:

* Contribution No. 2569 from the Department of Chemistry, U.C.L.A., U.S.A.

† Where it is undoubtedly adventive, of European origin.

‡ I am indebted to Dr. H. C. Friedrichs, University of Munich, Germany, for authenticating the material used.

[1] D. D. KECK, *Proc. Calif. Acad. Sci.* **25**, 421 (1946).
[2] T. A. GEISSMAN and G. A. ELLESTAD, *J. Org. Chem.* **27**, 1855 (1962).
[3] S. MATSUEDA and T. A. GEISSMAN, *Tetrahedron Letters* **1967**, 2159.
[4] S. MATSUEDA and T. A. GEISSMAN, *Tetrahedron Letters* **1967**, 2013.
[5] K. H. LEE and T. A. GEISSMAN, *Phytochem.* in press.
[6] T. A. GEISSMAN, *J. Org. Chem.* **31**, 2523 (1966).
[7] G. HEGI, *Illustra. Flora Mittel-Europa* (2nd edition), Lehmann's Verlag, Munich (1935).

artemorin (II), verlotorin (III), and anhydroverlotorin (dehydroartemorin) (IV).* No vulgarin was present.

Artemorin, $C_{15}H_{20}O_3$, is a hydroxylactone. Its i.r. and u.v. spectra indicated that it contained the α-methylene-γ-lactone grouping typical of many of the compounds of the class. This conclusion was substantiated by the NMR spectrum, which was readily interpretable as corresponding to structure II.[8] A pair of doublets ($J = 3$ Hz) at δ 5·44 and 6·18 are characteristic of the C-11/13 methylene grouping. A 1-proton triplet ($J = 7$ Hz) at δ 3·99, with each peak broadened by allylic coupling, corresponds to C*H*OH at C-1. This signal is missing in artemorin acetate (V), where it is replaced by a 1-proton signal (not clearly resolved) at δ 5·20. The proton of the lactone grouping at C-6 appears as a well-defined triplet ($J = 10$ Hz) at δ 4·40, coupled with the vinyl proton at C-5, which appears as a doublet ($J = 10$ Hz) at δ 5·23. Artemorin contains but one methyl group, seen as a doublet ($J = 1$ Hz) at δ 1·71, clearly a methyl group in the system $CH_3C{=}CH$. The two protons of the methylene group at C-10 appear as two broadened singlets at δ 4·88 and δ 5·20. The signals for the methylene group at C-10, and the protons at C-5 and C-6 are nearly identical in form and position with those of ridentin (VI).[8] Integration of the NMR spectrum provided an accurate count of the twenty protons required by the molecular formula.

Artemorin acetate (V) shows all of the expected signals in its NMR spectrum, most of them similar in form and position to those of II, and in addition a 3-proton singlet for the acetyl methyl group at δ 1·98.

A most revealing property of artemorin (or its acetate) is the formation of a deep color when to its ethanolic solution is added a few drops of conc. HCl. The solution, colorless when cold, becomes rose-red on warming and upon further gentle heating a deep burgundy-red. The behavior and appearance of the color are almost exactly like those seen with xanthinin[9] when it is treated in the same manner.[10] This observation is in accord with the structure II, for acid-catalyzed ring closure of artemorin across the C-1/C-5 positions can give rise to the guaianolide system required[10] for the formation of the colored product.

Verlotorin (III), $C_{15}H_{20}O_4$, shows many of the same structural features as artemorin: the i.r. and u.v. spectra are almost identical, the i.r. spectrum of verlotorin differing significantly only in the OH region. The NMR spectrum of verlotorin shows signals corresponding to the α-methylene grouping of the lactone (two doublets, 1 proton each, δ 5·47 and 6·18, $J = 3$ Hz); the vinyl methyl group at C-4 (3-proton doublet, δ 1·69, $J = 1$ Hz); the C-6 proton (triplet, δ 4·38, $J = 10$ Hz); and the methylene group at C-10 (two broadened 1-proton singlets, δ 5·06 and 5·22). The region in which the C*H*OH signals of two secondary hydroxyl groups are to be expected contains 2 protons, but these are not clearly resolved and neither is separately discernible; and the expected 1-proton doublet (with $J = 10$) for the C-5 proton is seen at δ 5·30, 0·5 of the doublet, however, being obscured. The number and chemical shifts of the protons in the region δ 3–δ 6 is such as to support the conclusion that verlotorin contains two secondary hydroxyl groups.

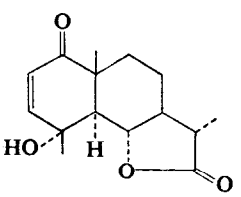
* Anhydroverlotorin is possibly an artefact derived from verlotorin (*vide infra*) during the extraction and purification process. It was, however, identifiable as a constituent of the plant extractives before extensive manipulation.

[8] Certain features of the NMR spectrum were nearly the same as some of those shown by ridentin (VI), a lactone closely similar in its general structure. See M. A. IRWIN, K. H. LEE, R. F. SIMPSON and T. A. GEISSMAN, *Phytochem.* **8**, 2009 (1969).

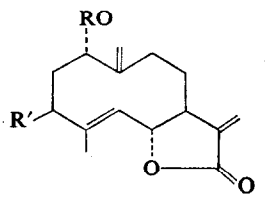
[9] T. A. GEISSMAN, *J. Org. Chem.* **27**, 2692 (1962).

[10] For further comment on this color reaction see A. YOSHITAKE and T. A. GEISSMAN, *Phytochem.* **8**, 1753 (1969).

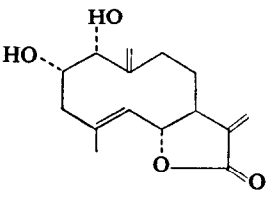
An attempt to acetylate verlotorin led to an illuminating and significant result. Treatment of verlotorin with pyridine–acetic anhydride in the usual way resulted in the formation of a crystalline compound. This was not an acetate, and its composition ($C_{15}H_{18}O_3$) was that of a dehydration product of verlotorin. The same compound was isolated by chromatography of the extract of the plant. Its formulation as IV is clearly supported by its composition and spectral characteristics. The compound showed prominent i.r. absorption at 1751 cm^{-1} (lactone) and 1660 cm^{-1} (α,β-unsaturated ketone). Its NMR spectrum is in excellent accord with IV. Besides the signals for the C-4 methyl group (3 H, doublet, δ 1·77, $J = 1$ Hz) and an unresolved complex (9 H, δ 2·0–3·2), there are seen six well-separated and clearly resolved 1-proton signals at lower fields. A triplet at δ 4·34 ($J = 10$ Hz) corresponds to the C*H*—O proton of the lactone ring at C-6. The vinylic proton at C-5 is seen as a doublet at δ 5 10 ($J = 10$ Hz) and the protons of the α-methylene group at C-11/C-13 appear as the characteristic doublets ($J = 3$ Hz) at δ 5·50 and 6·23. The remaining 2 protons of the C-10 methylene group are seen as two sharp singlets at δ 5·69 and 5·84; their displacement downfield (from δ 5·06 and 5·22 in verlotorin, and 4·88 and 5·20 in artemorin) is in accord with their situation in the α,β-unsaturated ketone system of IV. An alternative structure for anhydroverlotorin, with the newly generated carbonyl group at C-2 instead of C-1, would be expected to show carbonyl absorption in the i.r. in the 1700–1710 cm^{-1} region, and NMR signals for the C-10 methylene group at chemical shifts similar to those seen for this grouping in artemorin and verlotorin. Anhydroverlotorin no longer gives the red color with HCl that characterizes artemorin.*



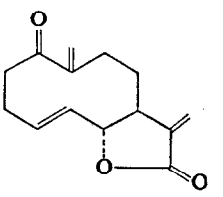
(I)



(II) R′ = R = H
(V) R′ = H; R = Ac
(VI) R = H; R′ = OH (RO-β)



(III)



(IV)

The formation of IV from III evidently occurs by a pinacol rearrangement and shows that verlotorin possesses to adjacent hydroxyl groups.

Two observations lead to the assignment of the stereochemistry as shown in II and III.† In the first place, the only significant differences between the i.r. spectra of II and III are seen in the region of hydroxyl absorption. Artemorin shown a peak at 3510 cm^{-1}, while verlotorin shows a broadened, more intense absorption at about 3560 cm^{-1}, an indication of the presence of the hydrogen-bonded hydroxyl groups at C-1 and C-2. Equally significant is the behavior of the compounds in TLC. Artemorin, the monohydroxy compound, shows a lower R_f than verlotorin, the dihydroxy compound. This suggests that the hydroxyl group of artemorin is equatorially disposed in a ring conformation that permits it to extend from the molecule. Indeed, a model of the compound in this conformation easily places the other

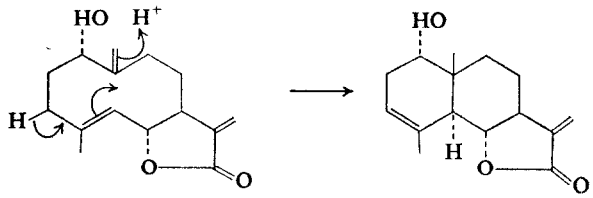
* Verlotorin gives a detectable but fleeting color in this test, indicating that the structural requirement for the color reaction is satisfied but that alternative changes take place with greater ease.

† The stereochemistry of the lactone ring is shown by the NMR spectra, with the assumption that the C-7/C-11 bond is β-disposed.

substituents in positions consonant with the NMR signals. In particular, the large coupling constant shown by the C-1 proton is in accord with its axial nature. The axial–axial disposition of C-1/H and C-2/OH accounts for the otherwise unexpected fact that dehydration of verlotorin occurs by the loss of the axial OH at C-2 and H at C-1, rather than of the allylic but equatorial OH at C-1 and H at C-2.

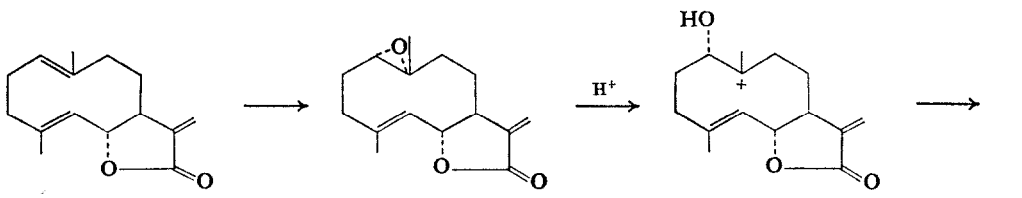
The biosynthetic relationships disclosed by the constituents of the several species of the *vulgaris* complex that have been investigated deserve comment. Studies of the species mentioned above[2–6] have led to the isolation of a number of closely related lactones. Some of the implications of these observations have been discussed elsewhere.[5] For the present discussion, douglanine (VII)[3] will be used as the example, for it has been found that this can be assigned to an early position in a sequence leading to arglanine[4] and to a group of compounds found in *A. ludoviciana*.[5]

It will be seen that douglanine (VII) can be related to artemorin by a simple and straightforward acid-catalyzed ring closure. The known[11] α-configuration of the C-1 hydroxyl group in douglanine is, of course, in agreement with this hypothesis of artemorin possesses the α-C-1 hydroxyl group shown in II:



(II) (VII)

Moreover, since it can be assumed that the early precursors of the sesquiterpenoid lactones are the cyclodecadienolides derived by way of farnesol cyclization, it can be suggested that artemorin represents an early stage in the overall synthetic pathway leading to the santanolides of the *vulgaris* group. The widespread occurrence of epoxides among the members of some series of co-occurring lactones[5,12] leads to the suggestion that epoxidation is an important route of entrance of oxygen into natural organic compounds. This leads to the rational suggestion that artemorin may have its origin in a manner such as the following:[13,*]



Constunolide (VIII) (IX) (II) ⟶ (VII)

* Costunolide occurs in several genera of the Compositae, including an *Artemisia* spp.

[11] M. T. EMERSON, unpublished work.
[12] For example, T. G. WADDELL and T. A. GEISSMAN, *Phytochem.* **8**, 2371 (1969).
[13] It is to be recalled that pyrethrosin, the 8-acetoxy derivative of IX, occurs in a *Chrysanthemum* spp.: D. H. R. BARTON and P. DE MAYO, *J. Chem. Soc.* 150 (1957).

EXPERIMENTAL

Artemisia verlotorum Lamotte was collected in Milbertshofen, North Munich, Germany. The leaves were stripped from the stems,* dried in air, ground to a powder (2·1 kg) and extracted exhaustively with $CHCl_3$. Removal of the solvent left a green-black tar which was dissolved in 200 ml of hot ethanol. After the addition of 400 ml of hot water the tarry residue was separated from the yellow aqueous solution and reextracted with several portions of hot 50% aq. ethanol. The aqueous extract was clarified by filtration (celite, charcoal) and extracted exhaustively with $CHCl_3$. Removal of the $CHCl_3$ left a yellow-brown oil. This was dissolved in ether and washed with cold 0·5 N NaOH; water, dilute HCl, water, and finally dried and evaporated. There remained 15·6 g of a deep yellow oil.

This oily material was placed upon a silica gel column (450 g; 6 × 40 cm) and eluted with $CHCl_3$ (25 × 100 ml + 1 × 500 ml) and finally with 1000 ml of 5% MeOH in $CHCl_3$ (fraction 27).

Verlotorin (*III*). From fractions 16–23 were obtained 1·44 g of crystalline verlotorin, which was purified by a second passage through silica gel ($CHCl_3$). With the addition of more material obtained by chromatography of verlotorin-containing mother liquors, the total yield of purified verlotorin was 1·38 g. It formed colorless prisms which, when heated slowly (Kofler stage), grew yellow but did not melt below 240°. When placed on the preheated stage and heated rapidly, it melted in the range 130–132°; when put on the stage at 150° it melted at once to a colorless liquid. Calc. for $C_{15}H_{20}O_4$: C, 68·2; H, 7·6; found, C, 68·1; H, 7·5,

Anhydroverlotorin (*from plant extract*) (*IV*). Early fractions collected during the chromatographic purification of verlotorin yielded 70 mg of a crystalline compound, m.p. 123–124°. This proved to be identical (mixed m.p. NMR) with the compound formed in the reaction of verlotorin with acetic anhydride–pyridine (see below).

Artemorin (*II*). Fraction 27 ($CHCl_3$–5% MeOH) from the original column was evaporated to an oil which was chromatographed over silica gel ($CHCl_3$). From an early fraction was obtained 50 mg of anhydroverlotorin (IV). Later fractions yielded 910 mg of artemorin. Recrystallized from CH_2Cl_2–Et_2O, it formed glistening leaflets, m.p. 115–117°. Calc. for $C_{15}H_{20}O_3$: C, 72·6; H, 8·1; found, C, 72·6; H, 8·0.

On silica gel (TLC) with benzene–acetone (3:1) as solvent, the R_f values of anhydroverlotorin, verlotorin and artemorin were, respectively, about 0·65, 0·50 and 0·35. Both verlotorin and artemorin show immediate orange-brown colorations when the plate is sprayed with concentrated H_2SO_4. When the sprayed plate is warmed the artemorin spot becomes purple; the verlotorin spot darkens to an indefinite brown. Both verlotorin and artemorin are unstable on keeping at ordinary temperatures.

Anhydroverlotorin. A solution of 1·07 g of verlotorin in a mixture of 2 ml of pyridine and 3 ml Ac_2O was kept overnight. Ice and ether were added and the ether layer was washed with dilute $NaHCO_3$, dilute HCl and water, and evaporated. The addition of a little pentane to the oily residue caused immediate crystallization. Recrystallized from CH_2Cl_2–Et_2O, the compound formed glistening, colorless leaflets, m.p. 123–124°.

The compound was identical with the material isolated by chromatography of the plant extract (see above). Calc. for $C_{15}H_{18}O_3$: C, 73·2; H, 7·3; found: C, 73·4; H, 7·2.

Artemorin acetate (*VI*) was prepared in the usual way with pyridine–Ac_2O. Crystallized from Et_2O–CH_2Cl_2, it formed shining, colorless leaflets, m.p. 115–116°. Its NMR and i.r. spectra established that it was the expected monoacetate. Artemorin acetate and anhydroverlotorin have nearly identical R_f on TLC, but are readily distinguished by the purple color of the spot of the acetate when the H_2SO_4-sprayed plate is warmed. Anhydroverlotorin gives no color with H_2SO_4, and on heating the spot develops a brown or black color.

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* Separate examination of stem material showed that it contained very little extractable material.