

## SESQUITERPENE LACTONES OF *ARTEMISIA* SPECIES: ARTEFRANSIN FROM *A. FRANSERIOIDES*

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**Abstract**—Artefransin, a new guaianolide from *Artemisia franserioides* Greene, has been shown to have the structure I. Artefransin bears a close structural relationship to ligustrin and the cumambrins.

### INTRODUCTION

AS A PART of the continuing study of the chemical contributions to taxonomy and phylogeny in the genus *Artemisia*, we have examined the sesquiterpenoid constituents of *A. franserioides* Greene, a member of the Section Abrotanum, several species of which have already been studied in this laboratory. *A. franserioides* yielded but one sesquiterpene lactone, artefransin, in very small amount, the structure and biosynthetic relationships of which to other lactones are the subject of this communication.

### RESULTS AND DISCUSSION

Artefransin (I), the principal lactonic constituent of the mature plant,<sup>1</sup> was isolated in about 0.003% yield. The compound, m.p. 197–198°,  $[\alpha]_D^{25} + 33^\circ$ , analyzed for  $C_{17}H_{20}O_6$ , a result confirmed by the appearance of the molecular ion peak at  $m/e$  320 in the mass spectrum. The u.v. (206 nm,  $\epsilon$  10,000) and i.r. spectra (1765 and 1650  $cm^{-1}$ ) indicated the presence of the  $\alpha$ -methylene- $\gamma$ -lactone grouping common to this class of compounds. An i.r. band at 1720  $cm^{-1}$  suggested the presence of an acetoxy grouping, and an NMR signal at  $\delta$  2.09 (3 H singlet) and a mass spectral peak at  $m/e$  260 (M-18-42) established the presence of this structural feature. The presence of a secondary hydroxyl group was shown by the presence of an i.r. band at 3450  $cm^{-1}$ , a signal at  $\delta$  3.77 (1 H, d,  $J = 4.5$  Hz) in the NMR which disappeared on addition of  $D_2O$ , an ion at  $m/e$  302 (M-18) in the mass spectrum, and the formation of an acetate (the diacetoxyl compound II),  $C_{19}H_{22}O_7$ . The NMR spectrum of II showed the two acetyl methyl groups as sharp singlets at  $\delta$  2.11 (3 H) and 2.02 (3 H).

The presence of the epoxy grouping at C-10/C-14 was first suggested by the appearance of i.r. bands at 3060, 3045, 1265, 1255 and 1150  $cm^{-1}$ , and substantiated by the presence in the NMR spectrum of a sharp two-proton singlet at  $\delta$  2.80. This signal was substantially the same in the NMR spectrum of the acetate (II). Artefransin gave a positive result in the test for the epoxide function.<sup>2</sup> The exocyclic disposition of the epoxide grouping was established by treatment of artefransin with potassium selenocyanate with the formation of a

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<sup>1</sup> Studies on the relationship of the degree of maturity of a plant and its chemical constitution have shown that there can be gross differences between seedling (or young) and fully developed plants. For example, see T. A. GEISSMAN, T. S. GRIFFIN, T. G. WADDELL and H. H. CHEN, *Phytochem.* **8**, 145 (1969). Recent (unpublished) work by the present authors has shown that this is true also in the case of *A. douglasiana* Bess.

<sup>2</sup> J. M. ROSS, D. S. TARBELL, W. E. LOVETT and A. D. CROSS, *J. Am. Chem. Soc.* **78**, 4675 (1956).

precipitate of selenium. Ludovicin-A (III) a santanolide containing a 3,4-epoxy grouping, gave a negative result in this test.<sup>3</sup>

The most conspicuous feature of the NMR spectrum of artefransin (I) is the absence of methyl signals (except for that of the acetyl group). The  $\alpha$ -methylene- $\gamma$ -lactone is revealed by the characteristic pair of low-field doublets ( $\delta$  5.72, 6.12;  $J = 3$  Hz), neither of which shows geminal coupling.<sup>4</sup> The *trans*-axial relationship between the protons at C-5, C-6 and C-7, with H-5 $\alpha$ , H-6 $\beta$ , H-7 $\alpha$ , is seen in the signal for H-6, which is a well-defined one-proton quartet at  $\delta$  4.30 ( $J = 9.0, 10.5$  Hz), a feature common to most of the guaianolides (and santanolides) of *Artemisia*. The proton (at C-8) of CH-OAc is seen as a multiplet at  $\delta$  5.09 (1 H), which was unchanged in the acetate (II). The methylene group at C-4 in I appears as a double quartet at  $\delta$  5.41 and 5.34 (each 1 H,  $J = 3.0, 1.5$  Hz).

The secondary hydroxyl group is placed at C-3. The proton at C-3 (of CHOH) appears as a multiplet at  $\delta$  4.52 (1 H) is shifted to  $\delta$  5.54 in II, and the effect of acetylation of C-3 OH is seen in the shift of the signals for C-4 (=CH<sub>2</sub>) from  $\delta$  5.41 and 5.34 in I to  $\delta$  5.61 and 5.46 in II. That the secondary hydroxyl group is at C-3 and not C-9 (with corresponding changes in the position of the epoxide and exocyclic methylene groups at C-10 and C-4) can be inferred from the appearance of the CHOH-CHOAc protons in I and II. The latter are multiplets, clearly coupled with the three protons at C-7 and C-9.

The foregoing evidence leads to the assignment of structure I to artefransin, in which some of the stereochemical assignments remain to be established. The structure of artefransin bears a suggestive relationship to those of ligustrin (IV)<sup>5</sup> and the cumambrins (A, VI; B, V).<sup>6,7</sup> The process of oxidative elaboration, principally by way of epoxidation, in these guaianolides can be represented by the process shown in the suggested transformation IV  $\rightarrow$  VII  $\rightarrow$  I. In other work in progress in this laboratory we have observed the co-occurrence of an oxide (corresponding to the 3,4-epoxide VII) and the related unsaturated alcohol (corresponding to I) in the same plant.

Although the stereochemistry of I is not established in all details, biogenetic relationships (especially those described above) suggest that 3-OH is  $\alpha$  and 8-OAc is  $\alpha$ . The configuration of the C-10 grouping remains in doubt.

#### EXPERIMENTAL

Melting points were determined in capillary tubes and are corrected. U.v. spectra were determined in 95% ethanol. I.r. spectra were determined in Nujol mulls. NMR spectra were determined on a Varian Associates HA-100 instrument using tetramethylsilane as the internal standard; *s* refers to singlet, *d* to doublet, *q* to quartet and *m* to multiplet. Mass spectra were determined on an A.E.I. MS-9 instrument at 70 eV using direct insertion. Silica gel for column chromatography refers to Baker A.R. No. 3405 and silica gel for TLC refers to Merck silica gel G, developed with benzene-ethyl acetate (1:1) and visualized by spraying with conc. H<sub>2</sub>SO<sub>4</sub> and heating.

#### Extraction of *Artemisia franserioides* Greene

*A. franserioides* Greene was collected in September 1968 along shaded, rocky slopes above Benny Creek near Greer at 8200 ft elevation in Apache County, Arizona.<sup>†</sup> The dried, milled plant (2.2 kg) was exhaustively

\* See Ref. 2; from *Ambrosia acanthicarpa*.

<sup>†</sup> Collected and identified by Mr. R. J. Barr, Tucson, Arizona; Voucher No. 68620.

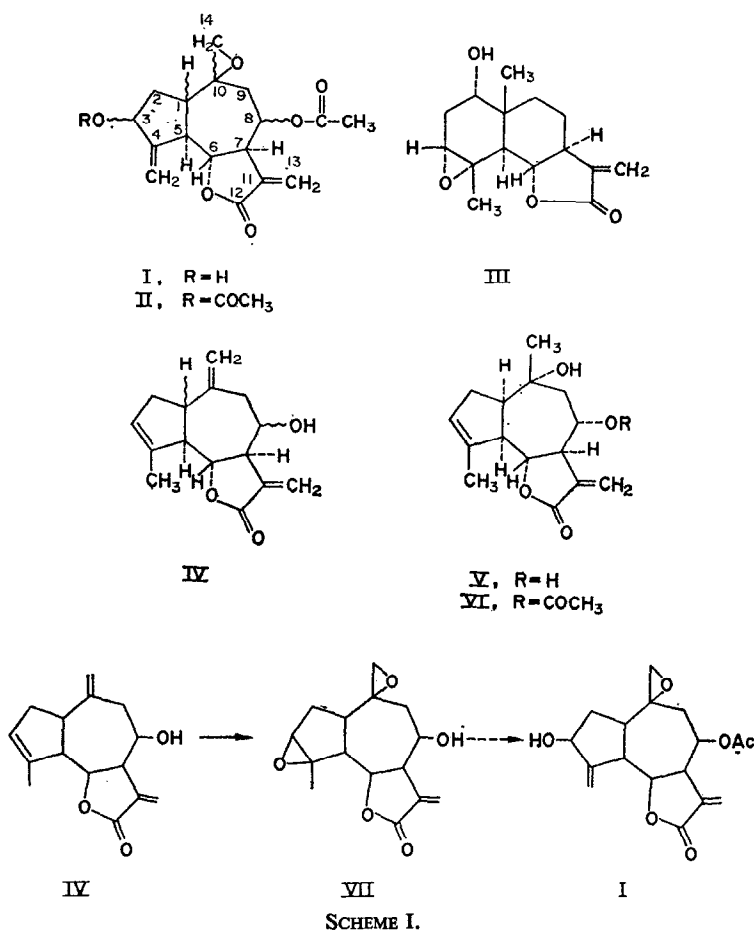
<sup>3</sup> C. C. J. CULVENOR, *Australian J. Chem.* **17**, 233 (1964).

<sup>4</sup> A study of the geminal coupling of the C-13 protons in lactones of this class is to be published: H. YOSHIOKA, T. J. MABRY, M. A. IRWIN, T. A. GEISSMAN and Z. SAMEK.

<sup>5</sup> J. ROMO, T. RIOS and L. QUIJANO, *Tetrahedron*, **24**, 6089 (1968); from *Eupatorium ligustrinum*.

<sup>6</sup> J. ROMO, A. ROMO DE VIVAR and E. DIAZ, *Tetrahedron*, **24**, 5625 (1968); from *Ambrosia cumanensis*.

<sup>7</sup> M. A. IRWIN and T. A. GEISSMAN, *Phytochem.* **8**, 305 (1969); from *Artemisia nova*; *A. tripartita* ssp. *tripartita*; *A. tripartita* ssp. *rupicola*.



extracted with  $\text{CHCl}_3$  at ordinary temperature. After removal of the solvent the tarry residue was slurried with methanol (3000 ml) and shaken with *n*-hexane (2000 ml) and water (1000 ml). The aqueous layer was washed with hexane and the hexane layer was extracted with water. The combined aqueous extracts were concentrated *in vacuo* and extracted again with  $\text{CHCl}_3$ . The total  $\text{CHCl}_3$  extract was washed with water, dried over anhydrous  $\text{K}_2\text{CO}_3$  and evaporated under reduced pressure to yield a dark greenish-brown syrup (32 g).

#### Isolation of Artefransin (I)

The above crude syrup (32 g) was dissolved in  $\text{CHCl}_3$  and chromatographed on silica gel (450 g). Elution was carried out successively with solvents ranging from  $\text{CHCl}_3$  to a final EtOAc-acetone (1:1) mixture. Twenty-six 500-ml fractions were collected and the eluted fractions were examined by TLC. The fractions obtained from  $\text{CHCl}_3$  (1-6),  $\text{CHCl}_3$ -EtOAc (9:1) (7-9) and  $\text{CHCl}_3$ -EtOAc (6:1) (10) eluates were combined and rubbed with ether to give 90 mg of a crystalline residue which was recrystallized three times from  $\text{CH}_2\text{Cl}_2$  to provide colorless prisms, m.p. 267-269°. The TLC, however, showed that this substance is a mixture of two closely related compounds with the approximate ratio of 1:1. An attempt to separate this mixture by rechromatography on silica gel (1.5  $\times$  17 cm) with hexane-EtOAc (1:1) to a final EtOAc-acetone (6:1) mixture as eluants has not yet been successful. This mixture remains to be investigated in detail.

The subsequent fractions obtained from  $\text{CHCl}_3$ -EtOAc (6:1, 3:1 to 1:1) (11, 12-14 to 15-17) and EtOAc (18) were combined and concentrated *in vacuo*, and the crystals formed were recrystallized three times from EtOAc- $\text{CH}_2\text{Cl}_2$  to give artefransin (I) as fine colorless needles (65 mg), m.p. 197-198°,  $[\alpha]_D^{25} + 33^\circ$  (C = 0.6, pyridine). (Found: C, 63.75; H, 6.24. Calc. for  $\text{C}_{17}\text{H}_{20}\text{O}_6$ : C, 63.74; H, 6.29%). The relevant

features of the u.v., i.r., NMR and mass spectra are noted and described in the Results and Discussion section.

The polar fractions obtained from EtOAc and ETOAc-acetone mixture (19-26) as dark brown syrup will be reserved for further investigation.

#### *Artefransin Acetate (II)*

Artefransin (30 mg) was acetylated with pyridine-acetic anhydride in the usual way. The product was purified by passage over silica gel ( $1.5 \times 8.5$  cm). A homogeneous fraction (by TLC) in  $\text{CH}_2\text{Cl}_2$  eluate was crystallized from  $\text{Et}_2\text{O}-\text{CH}_2\text{Cl}_2$  as fine colorless needles (II), m.p.  $154-155^\circ$ . It showed an end absorption in the u.v. spectrum at 210 nm and i.r. (in  $\text{CHCl}_3$ ) bands at 1765, 1660 ( $\gamma$ -lactone- $\alpha$ -methylene), 1735 (dimeric, two acetyl carbonyl) and 1140 ( $-\text{C}-\text{O}-\text{C}-$ ), and the absence of the hydroxyl absorption. The mass spectrum showed the molecular ion at  $m/e$  362 and the prominent peaks at  $m/e$  320 (M-42), 302 (M-42-18), 260 (M-42-18-42) and 242 (M-42-18-42-18). (Found: C, 63.07; H, 6.24. Calc. for  $\text{C}_{19}\text{H}_{22}\text{O}_7$ : C, 62.97; H, 6.12%).

#### *Treatment of Artefransin with Potassium Selenocyanate*

A solution of artefransin (1 mg) and potassium selenocyanate (1 mg) in 50% aq. ethanol (0.2 ml) was heated under reflux. A red amorphous precipitate of selenium complex formed within one hour indicating a positive result for the test of the epoxy function as shown in I. When ludovicin-A was treated in the same way the solution remained clear and colorless.

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