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SESQUITERPENE LACTONES OF *ARTEMISIA*: NEW GUAIANOLIDES FROM FALL GROWTH OF *A. DOUGLASIANA**

K. H. LEE, S. MATSUEDA and T. A. GEISSMAN
Department of Chemistry, University of California, Los Angeles, California 90024, U.S.A.

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Abstract—In contrast to spring growth, in which only santanolides were found, mature, flowering fall growth of *Artemisia douglasiana* Bess, contains guaianolides. Two new lactones, arteglasin-A and -B, have been isolated and their structure established. It is possible to relate the structures of the santanolides and the guaianolides by a simple change in early stages of a rational course of biosynthesis.

INTRODUCTION

Artemisia douglasiana Bess. (Section Abrotanum, Compositae), is a perennial abundant in Southern California. Examination of early spring growth showed that it contained the santanolides douglanine,[1] arglanine,[2] and ludovicin-B.[3] An early study of the fall plant in the flowering stage† disclosed that the lactones present at maturity were different from those in the spring plant, and that they possessed the characteristics of guaianolides.† The new lactones were not fully characterized at that time,‡ and have now been isolated and their structures established.

RESULTS AND DISCUSSION

Chromatography of the extract of *A. douglasiana* led to the isolation of four crystalline compounds. Two of these (C and D) were obtained in amounts too small to permit their thorough study. Arteglasin-A (I), obtained in the less polar fractions, m.p. 207–208°, has the composition $C_{17}H_{20}O_5$ and showed a molecular ion at *m/e* 304 in the mass spectrum. Its u.v. and i.r. absorption (204 nm, ϵ 11600; 1765, 1650 cm^{-1}) were indicative of the presence of the α-methylene-γ-lactone grouping. Mass spectral peaks at *m/e* 262 (M-42), 244 (M-60) and 202 (M-42-60), and i.r. absorption at 1740 and 1240 cm^{-1}, coupled with the C_{17} formula, showed that arteglasin-A was an acetate of a C_{15} compound, a conclusion verified by the appearance of a three-proton singlet for the acetyl methyl group at δ 2·08 in the NMR spectrum. The NMR spectrum showed the signals typical of the α-methylene-γ-lactone grouping (two doublets, each 1 H, δ 5·57 and 6·18, $J = 3$ Hz), and a one-proton triplet at

* Contribution No. 2612 from the Department of Chemistry, UCLA.

† The mature plant was that growing on the same rootstock from which the spring growth was collected. Thus, the differences found were neither varietal nor due to chemovariant populations.

‡ This observation was described in a lecture at the Second Natural Products Symposium, University of the West Indies, Mona, Jamaica, 2–5 January, 1968.

[1] S. MATSUEDA and T. A. GEISSMAN, *Tetrahedron Letters* 2159 (1967).
[2] S. MATSUEDA and T. A. GEISSMAN, *Tetrahedron Letters* 2013 (1967).
[3] K. H. LEE and T. A. GEISSMAN, unpublished observation.

δ 3·72 (J = 10 Hz) for the lactonic proton at C-6. The nature of this signal shows the *trans*-diaxial disposition of the protons at C-5 (α), C-6 (β) and C-7 (α), a feature common to most of the lactones of the genus. A sextet centered at δ 4·80 (J = 10, 10, 3 Hz) for the CH-OAc proton indicated that it was located at the expected (from biogenetic considerations) C-8 position.

The methyl group at C-4, attached to the C-3/C-4 epoxy grouping, is seen as a sharp singlet (3 H, δ 1·64), corresponding closely to the positions of the singlet signals for the C-4 methyl groups in canin (VIII)[4] and cumambrin-A epoxide (V),[5] which appear at δ 1·62 and 1·60 respectively. The C-3 proton of the C-3/C-4 epoxy grouping is seen as a broadened one-proton singlet at δ 3·38. This corresponds with the observation that the same proton in cumambrin-A epoxide appears as a singlet at δ 3·30.[5]

Since the NMR spectrum of arteglasin-A contains no low-field protons other than those for those of the C-13 methylene group and the C-6 lactonic proton, the vinylic methyl group (δ 1·72, 3 H, d, J = 1 Hz) must be found at C-10 and the double bond at C-1/C-10.

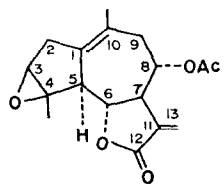
Epoxidation of arteglasin-A yielded the diepoxy compound, arteglasin-A epoxide (II), the C-10 methyl group of which appeared as a sharp 3-proton singlet at δ 1·37. This is almost identical in position with the signals for the corresponding methyl groups in arborescine (VI)[6] and globicin (VII),[7] both of which are seen as 3-proton singlets at δ 1·33. These observations lead to the expression I for arteglasin-A. The stereochemistry of the C-8 acetoxy group is not established by the above evidence, but because of the close structural relationship between arteglasin-A and other 8-hydroxy guaianolides found in *Artemisia* species it is suggested that the configuration of the acetoxy group is α. Although positive evidence for the configuration of the 8-hydroxyl group could not be obtained,* there is reason aside from the biogenetic argument, to believe that the orientation is 8α-OH. The coupling constants observed for the proton at C-8 (CHOAc) were 10, 10 and 3 Hz, showing an axial-axial disposition of H-8 with respect to H-7 and H-9ax. In cumambrin-B (IV), the observed geminal coupling of H-13a/H-13b shows that 8-OH is equatorial (and α-),[8] and fixes the conformation of the sevenmembered ring. If the 8-hydroxyl group of I is α, and the sevenmembered ring has the same conformation as that in cumambrin-B, a model shows that the 8-H occupies a position in which it is at 180° angles to both C-7 and C-9ax. It is to be remarked, however, that with the alternate conformation of the ring, with 8-OH β-disposed, a model shows that the 8-H is also *trans*-axial to H-7 and H-9. A final decision as to the matter can best be reached when 8-deacetyl arteglasin-A can be prepared. The presence or absence of geminal 13-Ha, b coupling will establish the configuration of 8-OH with certainty.[9]

Arteglasin-B (III), m.p. 192–4°, *m/e* 320 (M^+), has the composition $C_{17}H_{22}O_6$. Its u.v. and i.r. spectra indicated that it is an α-methylene-γ-lactone. The NMR spectrum confirmed this, and showed in addition a 3-proton singlet (δ 2·12) for the methyl group of an acetoxy grouping. Other NMR signals are seen at δ 1·65 (3 H, s; C-4 methyl), 3·60 (1 H, s; H-3), 3·94 (1 H, q, J = 11, 9 Hz; H-6), and 4·95 (1 H, m; H-8); these are nearly identical in form and multiplicity with the signals for the corresponding groups of I. The absence of a signal

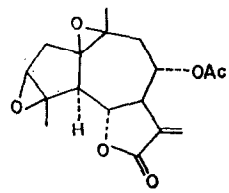
* Attempts to remove the acetyl group by selective hydrolysis in order to observe the geminal coupling of H-13a, b in the hydroxy compound, were unsuccessful. Hydrolysis under several conditions resulted in the formation of mixtures from which due to the limited amount of material, no pure product could be separated.

[4] K. H. LEE, R. F. SIMPSON and T. A. GEISSMAN, *Phytochem.* **8**, 1515 (1969).
[5] J. ROMO, A. ROMO DE VIVAR and E. DIAZ, *Tetrahedron* **24**, 5625 (1968).
[6] R. B. BATES, Z. CEKAN, V. PROCHAZKA and V. HEROUT, *Tetrahedron Letters* 1127 (1963).
[7] R. B. BATES, V. PROCHAZKA and Z. CEKAN, *Tetrahedron Letters* 877 (1963).
[8] H. YOSHIOKA, T. J. MABRY, M. A. IRWIN, T. A. GEISSMAN and Z. SAMEK, to be published.

for the C-10 methyl group in III, and the appearance of two broad one-proton singlets at δ 5·03 and 5·59 indicated that the C-10 position was occupied by an exocyclic methylene group. The additional oxygen atom in arteglasin-B is present in a tertiary hydroxyl group. This is inferred from an i.r. band at 3480 cm^{-1}, an ion at *m/e* 302 (M-18) in the mass spectrum, and an NMR signal at δ 4·03 (1 H, s) which disappeared when D_2O was added. Attempted acetylation failed. A consideration of these data lead to the conclusion that arteglasin-B has the structure III. It is apparent that the C-1 hydroxyl/C-10 methylene grouping could have resulted from the acid-catalyzed opening of a C-1/C-10 epoxy grouping, with the loss of the C-15 (CH_3) proton.



I



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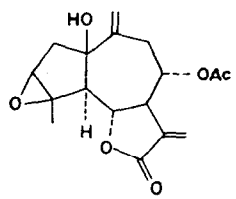
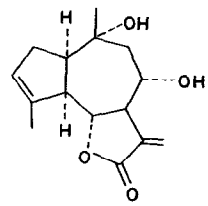


III



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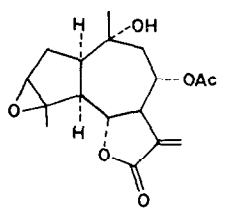
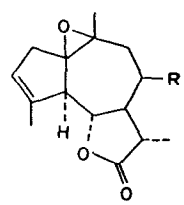
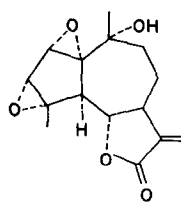


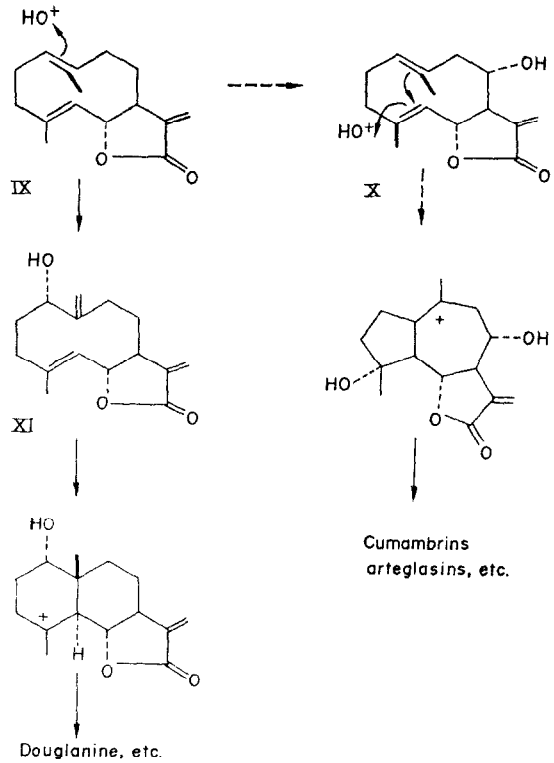
V



VI R=H
VII R=OAc



VIII



Attempts to convert arteglasin-A epoxide (II) into arteglasin-B (III) by acid-catalyzed opening of the C-1/C-10 epoxy grouping were unsuccessful, undoubtedly because of the sensitivity of the C-3/C-4 epoxy grouping to the conditions used. However, when II and III were treated in identical fashion in parallel experiments, TLC examination of the respective mixtures formed disclosed that both gave the same products.

The remarkable dissimilarity in the principal lactonic constituents of *A. douglasiana* in the spring and fall growth is suggestive of an alteration in the biosynthetic pathways that operate at different stages of growth. If it be assumed that the primary biosynthetic pathway undergoes no significant alteration, and, indeed, starts from a farnesol-derived germacranolide precursor, it can be suggested that the douglanine-arglanine pathway, and the arteglasin-A and -B pathway differ in a change in the initial position of attack of the oxidative cyclizing agent from which the bicyclic systems are derived. Taking costunolide (IX) as a prototype of the primary precursor, the sequences shown in Chart 1 can be suggested as those representing the two pathways of elaboration of the final products. Although the stage at which the 8-hydroxyl group is introduced is not known, in view of the common occurrence of 8-hydroxygermacranolides [e.g. (X)] it is possible that the presence of the hydroxyl group in the germacranolide precursor is the key to the alteration in the position of oxidative ring closure, perhaps by causing a change in the orientation of attachment of the substrate to the cyclizing enzyme.*

* The use of HO^+ is an arbitrary device which represents what in the natural process may involve an epoxidation.

The marked disparity in the composition of spring and fall *A. douglasiana* is not unique. It has been observed[10] that *Ambrosia acanthicarpa* seedlings also differ markedly from the mature plant. The relevance of these observations to chemotaxonomic applications of chemical data is obvious.

EXPERIMENTAL

M.ps were determined in capillary tubes, and are corrected. Optical rotations were determined in $CHCl_3$. U.v. spectra were determined in 95% EtOH. I.r. spectra were determined in Nujol mulls. NMR spectra were determined in $CDCl_3$ using tetramethylsilane as the internal standards; s, refers to singlet; d, to doublet; t, to triplet; 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–EtOAc (6:1) and visualized by spraying with conc. H_2SO_4 and heating.

Extraction of Fall Artemisia douglasiana

Fall *A. douglasiana* was collected in October 1969 in West Los Angeles. The dried and milled plant (6·83 kg) was exhaustively extracted with $CHCl_3$ at ordinary temperature, and the solvent removed to yield a tarry residue which was made slurried with methanol (3300 ml) and shaken with hexane (6000 ml) and water (1100 ml). The aqueous layer was washed with hexane and the hexane layer was washed with water. The combined aqueous extracts were concentrated *in vacuo* and extracted again with $CHCl_3$. The total $CHCl_3$ extract was washed with water, dried over Na_2SO_4 and evaporated under reduced pressure to yield a dark brown syrup (190 g).

Isolation of Arteglasins A (I), B (III), C and D

The crude extract (190 g) was chromatographed on silica gel (1·2 kg, 8·5 × 65 cm) and eluted successively with $CHCl_3$ containing increasing amounts of EtOAc, and then EtoAc containing increasing amounts of acetone. Forty 500-ml fractions were collected and the composition of the fractions determined by TLC. The first $CHCl_3$ eluate (fractions 1–2) contained only traces of waxes. The subsequent fraction (3) obtained from a $CHCl_3$ eluate contained mainly a single deep blue (H_2SO_4 spray) spot on TLC. The $CHCl_3$–EtOAc (1:1) (13–26) (1·1 g) and the EtOAc-acetone (27–40) eluates contained mainly a mixture of apparently two new guaianolides,* arteglasins-C and -D, which are currently under investigation.

Arteglasin-A (I). The fraction (3) obtained from the $CHCl_3$ eluate was rubbed with anhydrous Et_2O to yield colorless crystals (230 mg). Recrystallization from CH_2Cl_2–Et_2O afforded arteglasin A (I) as colorless scales, m.p. 207–208°, $[\alpha]_D^{25}$ + 110° (c = 0·8). The relevant spectral data u.v., i.r., NMR, M.S. have been described above. (Anal. Calcd for $C_{17}H_{20}O_5$: C, 67·09; H, 6·62. Found: C, 67·19; H, 6·73%)

Arteglasin-B (III). The combined $CHCl_3$ eluates obtained from fractions 4–12 were treated with a small amount of anhydrous Et_2O. The crude colorless crystals that formed were collected (1·66 g) and rechromatographed over silica gel (3 × 23 cm). Elution with benzene–Et_2O (6:1) provided a colorless crystalline material which was recrystallized repeatedly from acetone–benzene to give arteglasin B (III) as fine colorless needles, m.p. 192–194°, $[\alpha]_D^{25}$ + 150° (c = 0·5); i.r. bands at 3480 (OH), 1760, 1650 (γ-lactone-α-methylene), 1740 and 1245 (acetyl) cm^{-1} and an end absorption at 204 mμ (ε 12200) in the u.v. spectrum. (Anal. Calcd for $C_{17}H_{20}O_6$: C, 63·74; H, 6·29. Found: C, 63·66; H, 6·21%).

Arteglasin-A epoxide (II). A solution of arteglasin A (I) (30 mg) in $CHCl_3$ (1 ml) was treated with a solution of *m*-chloroperbenzoic acid (30 mg) in $CHCl_3$ (1 ml), and the mixture allowed to stand at room temp. overnight. The solution was washed with 5% aq. $NaHCO_3$ and with water, dried, and evaporated *in vacuo* to give colorless scales (II) m.p. 282–284°; i.r. bands at 1760, 1650 (γ-lactone-α-methylene), 1740 and 1240 (acetyl) cm^{-1}. (Anal. Calcd for $C_{17}H_{20}O_6$: C, 63·74; H, 6·29. Found: C, 63·54; H. 6·56%).

Attempted Acetylation of Arteglasin-A. A solution of arteglasin-A (5 mg) in Ac_2O (0·2 ml) and dry pyridine (0·4 ml) was kept at room temp. overnight. The product, recovered by working up the reaction mixture in the usual way, was the unchanged starting material (4 mg).

BF_3-Etherate treatment of arteglasin-A epoxide (II) and arteglasin-B (III). (A) A solution of arteglasin-A epoxide (II) (1 mg) in benzene (0·1 ml) and acetic acid (0·05 ml) was treated with BF_3-etherate (4 drops) and allowed to stand at room temp. overnight. The mixture was diluted with EtOAc, washed with 5% aq. $NaHCO_3$, water, dried and evaporated. This product was subjected to the TLC comparison with that of the same treatment of arteglasin-B.

* NMR spectra of this crystalline mixture indicated that they are guaianolides instead of santanolides or the other related sesquiterpene lactones.

[9] It is of interest to note that XI, called artemorin, has been isolated from another species of *Artemisia* belonging to the *vulgaris* group, which includes *A. douglasiana*; T. A. GEISSMAN, paper in press.

[10] T. A. GEISSMAN, T. S. GRIFFIN, T. G. WADDELL and H. H. CHEN, *Phytochem.* **8**, 145 (1969).

(B) Arteglasin-B was treated with BF_3-etherate and the product compared with TLC exactly in the same manner described for arteglasin-A epoxide. The products from A and B gave essentially the same mixture of products as shown by TLC.

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