Structures of the Artabsinolides; Photo-oxygenation Studies on Artabsin †

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The structure and stereochemistry of four new guaianolides from *Artemisia absinthium*, named artabsinolides A, B, C, and D, have been established. They were synthesized *via* rearrangement or reduction of the *endo*-peroxides resulting from dye-sensitized photo-oxygenation of the main constituent of the plant, artabsin (1).

SEVERAL types of sesquiterpene lactone have been isolated from *Artemisia absinthium*.[1] The major components are artabsin (1) [2] and various guaianolide dimers.[3-5] The other constituents belong to the germacrane [6] and eudesmane [7] series. We now report the structural elucidation of four new lactones, artabsinolides A—D, by correlation with artabsin (1).

Artabsinolide A (2) ($C_{15}H_{20}O_5$) is a hydroxy γ-lactone. Its i.r. and u.v. spectra indicated that it contained an α,β-unsaturated cyclopentenone. Its ^1H n.m.r. spectrum exhibits similarities to the spectrum of artabsin (1), showing a doublet (*J* 7 Hz) and two singlets due to a secondary and two tertiary methyl groups, respectively. An AB quartet (*J* 19 Hz) is assigned to the C-3 methylene protons, and a doublet (*J* 10 Hz) at δ 5.35 to H-6.

The ^{13}C n.m.r. spectrum of artabsinolide A could be readily interpreted on the basis of the planar structure (2). It shows absorptions due to 15 carbon atoms, assigned as two carbonyls, two fully substituted olefinic carbons, two oxygen-substituted quaternary carbons, three methines in the methyl lactone system, three methylenes, and three methyl carbons.

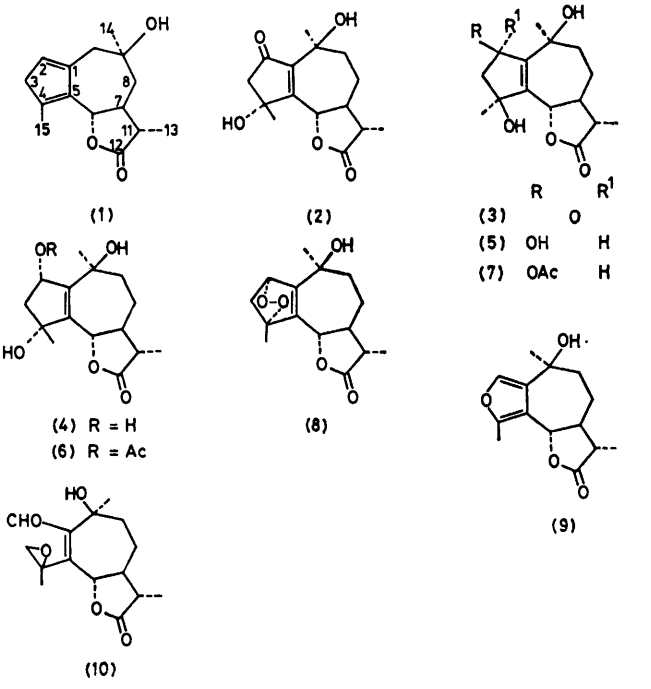
It was apparent from its spectral data that artabsinolide B (3) ($C_{15}H_{20}O_5$) is a stereoisomer of artabsinolide A (2). Their mass, i.r., and u.v. spectra are virtually identical and their ^1H and ^{13}C n.m.r. spectra show insignificant differences. These observations indicated that the two isomers might be C-4 epimers.

Artabsinolides C and D, contained in the more polar fractions of the plant extracts, could not be separated. Their purification was achieved through acetylation to give two isomeric monoacetates ($C_{17}H_{24}O_6$), assigned structures (6) and (7). Their i.r. spectra both show bands due to γ-lactone and acetyl functions.

The important feature of the ^1H n.m.r. spectrum of the acetyl derivative (6) is a triplet at δ 5.33, corresponding to $CH_2{\cdot}CHOAc$. De-*O*-acetylation of (6) yielded artabsinolide C ($C_{15}H_{22}O_5$) which, as expected, shows the *CH*OH signal at δ 4.80. Formation of the enone (2) on oxidation of artabsinolide C with Jones reagent strongly favours structure (4) for this new guaianolide.

The spectral data and the behaviour of acetyl derivative (7) were very similar to those of the acetyl derivative (6). It was transformed through the same reaction sequence (de-*O*-acetylation, oxidation) into artabsinolide B (3).

The structures and stereochemistries of artabsinolides A—D were further confirmed by correlation with artabsin (1). Thus, irradiation of a methanolic solution of artabsin (1) containing Rose Bengal, in the presence of

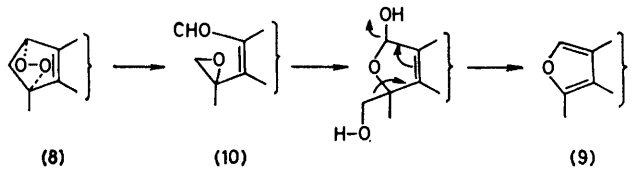


oxygen [8] gave the unstable *endo*-peroxide (8).[9] This derivative, which was the sole primary photo-oxidation product, could be isolated and characterized by n.m.r. spectrometry. Its ^1H n.m.r. spectrum showed a multiplet at δ 5.23 due to H-2 as well as the H-6 doublet and three methyl group signals. It decomposed slowly at room temperature to give three products in moderate yields.

The major decomposition product is a nor-sesquiterpene ($C_{14}H_{18}O_4$), assigned structure (9). Its ^1H n.m.r. spectrum exhibits H-2 and H-6 signals at δ 7 and 5.2, and three methyl resonances. The lowest field methyl singlet is found at δ 2.40, as expected for an α-methyl furan. The proposed structure (9) is fully supported by

† (11*S*)-10-Hydroxyguaia-1,4-dieno-6α,12-lactone.

the ^{13}C n.m.r. spectrum, which in particular shows the presence of four olefinic carbons. The mechanism depicted in the Scheme offers a reasonable explanation for the formation of (9). It is substantiated by the isolation of an unstable intermediate whose structure



SCHEME

(10) is in accord with its spectral data. Thus, the presence of an unsaturated aldehyde is indicated by a u.v. absorption band at 230 nm, and by a singlet at δ 10.2 in the n.m.r. spectrum. The latter shows the methylene proton AB quartet (J 5 Hz) of the epoxide ring at δ 2.80. When compound (10) was treated with silica gel it gave the furan (9).

The second product thermally derived from (8) is the diepoxide (11) ($M^{+\cdot}$ 180), whose structure was deduced from n.m.r. data and its chemical behaviour. In the ^1H n.m.r. spectrum the H-2 signal appears as a broad singlet at δ 3.68, and that of H-6 as a doublet at δ 5.23. On addition of shift reagent [Eu(fod)$_3$], the signals undergoing a low-field shift were those due to H-2, H-6, and the C-14 methyl as well as, to a lesser extent, that due to the C-15 methyl.* These observations suggest a complexation of europium with the β-oriented C-10 hydroxy-group, which induces deshielding of the protons on the β-side of the molecule. Therefore, the two epoxide rings should have the α-stereochemistry. This assignment is confirmed by the rearrangement of (11) in alkaline medium [10] to the secondary alcohol (12). The ^1H n.m.r. spectrum of (12) shows the H-2 doublet at δ 3.75, shifted to 4.92 on acetylation to give (13). Double-resonance experiments on compound (13) confirmed that the H-2 signal is the X part of an ABX system involving the methylene at C-3.

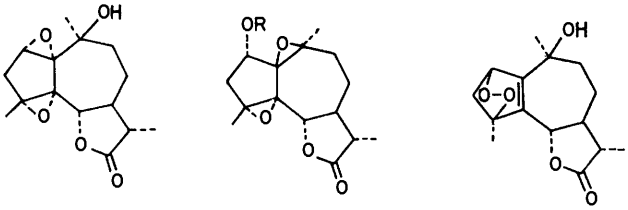
The formation of compound (12) demonstrates that in the parent derivative (11) the 1,2-epoxide ring and the 10-hydroxy-group are *trans*-oriented. Consequently, the initially formed *endo*-peroxide (8) must have resulted from α-attack of oxygen on the cyclopentadiene system of artabsin (1). Accordingly, the diepoxide which resulted from the isomerization of (8) could be given structure (11), in agreement with the europium-induced shift experiments.

The last product resulting from the thermal decomposition of the *endo*-peroxide (8) was identical with artabsinolide A (2). The same compound could be also obtained by treatment of (8) with alkali, thus confirming the α-stereochemistry of the 4-hydroxy-group in compound (2).[8]

* For H-2, H-6, C-14, and C-15 methyl protons the Δδ values were 0.15, 0.10, 0.08, and 0.06 p.p.m., respectively. We thank Mrs. C. Fontaine for this experiment.

Finally, as the reduction of the *endo*-peroxide (8) with thiourea yielded a triol identical with artabsinolide C(4), the former must have the two hydroxy-groups at C-2 and C-4 α-oriented.

An unexpected solvent effect was observed during the photo-oxygenation of artabsin (1). When this reaction was run in methylene chloride a 1 : 1 mixture of *endo*-peroxides (8) and (14) was obtained, as shown by the n.m.r. spectrum of the crude reaction mixture. It was not possible to separate these two *endo*-peroxides, but their reduction with thiourea led to a 1 : 1 mixture of artabsinolides C and D.



To summarise, this work has established unambiguously the structure and stereochemistry of four new guaianolides. They could be synthesized *via* rearrangement or reduction of the *endo*-peroxides (8) and (14) which resulted from the dye-sensitized photo-oxygenation of artabsin (1). As this reaction might mimic the initial step in the biogenetic pathway leading to artabsinolides, it is important to note that the major thermal degradation product of the *endo*-peroxides (8) and (14) was the furan (9), and that this stable compound was not isolated from the fresh plant extracts. Therefore, it is very unlikely that in *A. absinthium* artabsinolides A and B are formed spontaneously by a non-enzymic reaction of artabsin (1) with oxygen to produce an *endo*-peroxide. Finally, it is possible that such oxygenated derivatives, which have been found recently in several natural sources,[11] might play an important role in the oxidative functionalization of sesquiterpenes.[12]

EXPERIMENTAL

M.p.s were determined with a Kofler apparatus. Optical rotations were measured for solutions in $CHCl_3$. High- and low-resolution mass spectra were obtained with an A.E.I. M.S. 50 instrument. U.v. spectra were measured for solutions in 95% EtOH with a Bausch and Lomb Spectronic 505 spectrophotometer. I.r. spectra were recorded for solutions in $CHCl_3$ with a Perkin-Elmer 297 spectrophotometer. The ^1H n.m.r. spectra (Table 1) were obtained with a Varian EM-360L, a Bruker HX-90, or a Cameca 250 spectrometer, and the ^{13}C n.m.r. spectra (Table 2) with a Bruker HX-90 or WP-80 instrument for solutions in $CDCl_3$. Thin-layer chromatography was performed on Schleicher and Schüll silica gel F 1500 LS 254 plates.

Artemisia absinthium was collected in the Aosta valley (Italy). The blooming aerial parts were dried in air, ground to a powder, and extracted during 2 days with methylene chloride in the dark at room temperature. The residue

TABLE 1

^{1}H N.m.r. data (δ values)

Compound	H-2	H-3 [a]	H-6	H-13	H-14	H-15
(1)	6.00	2.81	5.31	1.20	1.55	2.15
(2)		2.70	5.35	1.20	1.50	1.75
(3)		2.70	5.40	1.25	1.50	1.70
(4)	4.80	2.38, 1.90	5.02	1.24	1.50	1.56
(5)	4.69	*b*	5.25	1.16	1.34	1.34
(6)	5.33	1.98	5.69	1.24	1.62	1.36
(7)	5.58	2.52, 1.98	5.48	1.24	1.48	1.38
(8)	5.23	2.07	5.00	1.20	1.43	1.82
(9)	7.0		5.20	1.20	1.60	2.4
(10)	10.2	2.8	5.21	1.20	1.50	1.60
(11)	3.68	1.77, 2.00	5.23	1.24	1.54	1.13
(12)	3.75	2.17, 2.33	4.36	1.20	1.50	1.70
(13)	4.92	2.47	4.40	1.21	1.30	1.70
(14)	5.40	*c*	4.96	*c*	1.41	1.78

[a] Centre of the AB system. [b] The spectrum of compound (5) was measured at 80 MHz; under these conditions the H-3 signals were obscured by other proton resonances. [c] Not assigned.

TABLE 2

^{13}C N.m.r. data (δ values)

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12	C-13	C-14	C-15	CO–CH_3
(1)	137.2	124	45.5	150.9	134	82	52.4	25.9	39.9	70.7	41.5	178.8	12.8	30.08	15	
(2)	143.7	206.6	51	75.4	166.1	76.3	47.8	23.8	35.8	71.7	42.6	176.4	12.8	27.2 [a]	26.9 [a]	
(3)	142.3	205.5	52.8	77.1	168.6	78.4	48.6	24.2	36.2	71.6	42.8	176.6	13	27.5 [a]	29.5 [a]	
(6)	145.5 [a]	76.4	46	82.2	143.1 [a]	79.4	48.9	24.5	38.7	71.6	42	177.4	12.6	28.4	27.4	178.4; 21.4
(7)	149.1 [a]	77	47.4	81.7	138.9 [a]	80.6	49.7	25.8	39.2	71.2	41.5	177.7	12.5	29.5 [a]	29 [a]	170.2; 21.4
(9)	130.4	136.6		147.2	117	76.7	52.9	25.6	40.2	69.3	41.5	178.9	12.5	30.2	13.3	
(11)	64.9 [a]	62.5	31.5	70.4	65.3 [a]	75.6	46.1	23.8	36	75.6	39.3	176.6	11	25.8	15	

[a] Assignments may be reversed.

obtained after filtration and removal of the solvent was re-extracted three times with hot 50% aqueous ethanol. The aqueous extract was clarified by filtration, concentrated, and extracted with CH_2Cl_2. Removal of the solvent gave a dark brown oil (30 g per kg of dried plant material).

The crude extract was chromatographed on silica gel. Elution with CH_2Cl_2 and CH_2Cl_2 containing an increasing proportion of methanol gave various products in the order and yields indicated in Table 3.

TABLE 3

Products from extraction of *A. absinthum*

Compound	Elution solvent	Yield [a]
Artemetin [13]	CH_2Cl_2—1.5% MeOH	4
Artabsin (1) [2]		2
3-Hydroxypelenolide [6a]		0.2
Artabsinolide A (2)		0.2
Isoabsinthin [5b]	CH_2Cl_2—2% MeOH	0.1
Absinthin [3]		3
Anabsin [4]	CH_2Cl_2—3% MeOH	0.6
Artabsinolide B (3)		0.2
Artabsinolide C (4) + Artabsinolide D (5)	CH_2Cl_2—10% MeOH	0.5

[a] In g per kg of plant material.

Artabsinolide A (2) had m.p. 160—162 °C (from ethanol); $[\alpha]_D$ +9° (*c* 0.1); $\lambda_{max.}$ 234 nm (ε 7 000); $\nu_{max.}$ 3 500 (OH), 1 780 (γ-lactone), and 1 690 cm^{-1} (CO); $M^{+\cdot}$ 280 (Found: C, 64.0; H, 7.2. $C_{15}H_{20}O_5$ requires C, 64.3; H, 7.2%).

Artabsinolide B (3) had m.p. 180—182 °C (from ethyl acetate–hexane); $[\alpha]_D$ +20° (*c* 0.1); $\lambda_{max.}$ 234 nm (7 000); $\nu_{max.}$ 3 500 (OH), 1 780 (γ-lactone), and 1 690 cm^{-1} (CO); $M^{+\cdot}$ 280 (Found: C, 64.5; H, 7.1. $C_{15}H_{20}O_5$ requires C, 64.3; H, 7.2%).

Fractions containing artabsinolides C and D (511 mg) were pooled and treated with acetic anhydride–pyridine overnight at room temperature. Silica gel column chromatography of the reaction mixture afforded *artabsinolide C acetate* (6) (143 mg) (eluted with hexane–30% acetone), m.p. 123—125 °C (from benzene–hexane); $\nu_{max.}$ 3 580 (OH), 1 780 (γ-lactone), and 1 735 cm^{-1} (ester); $M^{+\cdot}$ 324 (Found: C, 62.8; H, 7.3. $C_{17}H_{24}O_6$ requires C, 62.95; H, 7.45%) and *artabsinolide D acetate* (7) (158 mg) (eluted with hexane–35% acetone), m.p. 166—167 °C (from benzene–hexane); $\nu_{max.}$ 3 580 (OH), 1 780 (γ-lactone), and 1 740 cm^{-1} (ester); $M^{+\cdot}$ 324 (Found: C, 62.8; H, 7.2. $C_{17}H_{24}O_6$ requires C, 62.95; H, 7.45%).

Short treatment of compound (6) [or (7)] with NaOMe in methanol followed by neutralization gave artabsinolide C (4) [or artabsinolide D (5)]. Compounds (4) and (5) were purified by silica gel column chromatography (elution with CH_2Cl_2–6% MeOH) and obtained in yields of 25 and 50%, respectively. They were recrystallized from ethyl acetate.

Artabsinolide C (4) had m.p. 135—137 °C; $[\alpha]_D$ −22° (*c* 0.1); $\nu_{max.}$ 3 570, 3 400 (OH), and 1 770 cm^{-1} (γ-lactone) (Found: $M^{+\cdot}$ 282.1491. $C_{15}H_{22}O_5$ requires *M*, 282.1467).

Artabsinolide D (5) had m.p. 143—145 °C; $[\alpha]_D$ −51° (*c* 0.1); $\nu_{max.}$ 3 570, 3 450 (OH), and 1 780 cm^{-1} (γ-lactone) (Found: $M^{+\cdot}$ 282.1436. $C_{15}H_{22}O_5$ requires *M*, 282.1467).

Photo-oxidation of Artabsin (1).—A solution of artabsin (1) (496 mg, 2 mmol) in methanol (30 ml) containing Rose Bengal (50 mg) was irradiated under oxygen with visible light at −30 °C until t.l.c. (hexane–30% acetone) indicated

the complete disappearance of the starting material. The solution was kept overnight at room temperature or refluxed for 0.5 h, then evaporated, and the residue was chromatographed on a column of florisil (hexane–acetone gradient) to give (i) the *furan* (**9**) (152 mg, 30%), m.p. **145**—**147** °C (from benzene–hexane); $\nu_{max.}$ **3 500** (OH) and **1 760** cm^{-1} (γ-lactone) (Found: $M^{+\bullet}$, 250.1220. $C_{15}H_{18}O_4$ requires M, 250.1235); (ii) artabsinolide A (**2**) (25 mg, 5%); (iii) the *diepoxide* (**11**) (41 mg, 8%), m.p. 210—212 °C (from ethyl acetate–hexane); $\nu_{max.}$ **3 600** (OH) and **1 770** cm^{-1} (γ-lactone) (Found: C, **64.3**; H, **7.1**. $C_{15}H_{20}O_5$ requires C, **64.3**; H, **7.2**%); and (iv) artabsinolide B (**3**) (21 mg, 4%).

The *endo*-peroxide (**8**) could be obtained after photo-oxygenation in MeOH followed by evaporation at 0 °C. A part of the residue was soluble in $CDCl_3$ and gave a ^1H n.m.r. spectrum consistent with structure (**8**). Florisil column chromatography of this material gave various amounts of the aldehyde (**10**), $M^{+\bullet}$ **280**; $\lambda_{max.}$ 230 nm; $\nu_{max.}$ **3 450** (OH) and **1 770** cm^{-1} (γ-lactone).

The aldehyde (**10**) yielded the furan (**9**) upon warming or in the presence of silica gel.

Rearrangement of the Diepoxide (**11**).—To a solution of the diepoxide (**11**) (**70** mg) in dichloromethane (10 ml) was added cooled aqueous NaOH (0.5M; 10 ml). The mixture was stirred vigorously during 1 h at room temperature. After neutralization the product was extracted (CH_2Cl_2), to give after column chromatography (silica gel; hexane–acetone gradient) the *diepoxide* (**12**) (29 mg, 41%), m.p. **108**—**109** °C (from ethyl acetate–hexane); $\nu_{max.}$ **3 550** (OH) and **1 770** cm^{-1} (γ-lactone); $M^{+\bullet}$ **280** (Found: C, **64.05**; H, **7.2**. $C_{15}H_{20}O_5$ requires C, **64.3**; H, **7.2**%).

Acetylation of (**12**) (acetic anhydride–pyridine overnight) gave the *monoacetate* (**13**), m.p. 210—211 °C (from ethyl acetate–hexane); $\nu_{max.}$ **1 770** (γ-lactone) and **1 720** cm^{-1} (ester); $M^{+\bullet}$ **322** (Found: C, **63.5**; H, **6.7**. $C_{17}H_{22}O_6$ requires C, **63.35**; H, **6.9**%).

Treatment of the endo-*Peroxide* (**8**) *with Base*.—A methanolic solution (10 ml) of the *endo*-peroxide (**8**) prepared from artabsin (**1**) (100 mg, 0.4 mmol) following the foregoing procedure was treated with aqueous 9M-NaOH (5 ml) for 10 min at room temperature. The solution was carefully neutralized with aqueous HCl and extracted with CH_2Cl_2. Silica gel column chromatography of the reaction mixture (elution with CH_2Cl_2–MeOH gradient) gave artabsinolide A (**2**) (21 mg, 20%).

Reduction of the endo-*Peroxide* (**8**) *with Thiourea*.—Thiourea (**15** mg, 0.2 mmol) was added to a methanolic solution of the *endo*-peroxide (**8**) prepared from artabsin (**1**) (50 mg, 0.2 mmol). The mixture was stirred at room temperature during **36** h. The product was purified by silica gel column chromatography to give (elution with hexane–acetone gradient) artabsinolide C (**4**) (17 mg, **34**%).

Photo-oxidation of Artabsin (**1**) *in Dichloromethane*.—A solution of artabsin (**1**) (**595** mg, 2.4 mmol) in CH_2Cl_2 (30 ml) containing Rose Bengal (60 mg) in suspension was irradiated as above. Reduction of the mixture at room temperature with thiourea (**180** mg) during **36** h gave an equimolar mixture of compounds (**4**) and (**5**) which were eluted together from a silica gel column (with CH_2Cl_2–6% MeOH). Their acetates (**6**) (**56** mg) and (**7**) (**42** mg) were separated as indicated before.

A mixture of the *endo*-peroxides (**8**) and (**14**) was obtained after photo-oxygenation followed by filtration through a short column of Celite and evaporation at 0 °C. This mixture could be analysed by ^1H n.m.r. spectrometry (Table 1).

Oxidation of Artabsinolides (**4**) *and* (**5**). A solution in acetone (3 ml) of (**4**) [or (**5**)] (20 mg) was treated at 0 °C with Jones reagent. Filtration through Celite and chromatography gave (**2**) [or (**3**)] in 30% yield.

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