Evidence for the Biogenesis of 1α-Hydroxy-*trans*-eudesmanolides

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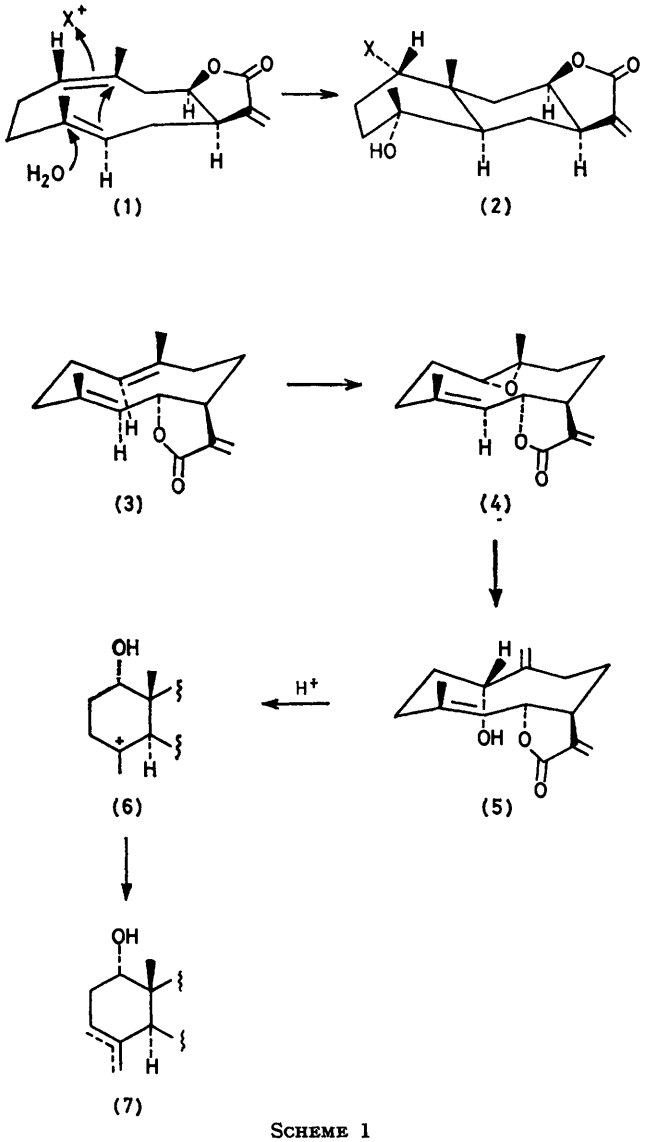
1-*epi*-Gallicin (10), a modified germacranolide, has been prepared from gallicin (12). A biogenetic-type cyclization of (10) afforded 1α-hydroxy-*trans*-eudesmanolides. The mechanism and the stereospecificity of the reaction are discussed in terms of a preferred reacting conformation. The possible biogenetic significance of the process is outlined.

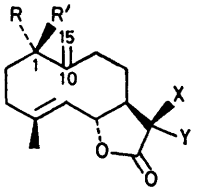
THE many stereospecific *in vitro* cyclization reactions of germacranolides and their epoxide derivatives have provided indirect evidence for the involvement of these compounds in eudesmanolide biosynthesis.[1] Parker, Roberts, and Ramage [2] suggested that the biogenesis of *trans*-eudesmanolides with α-oriented hydroxy-groups at C-1 may occur through the Markovnikoff-type *trans*-antiparallel cyclization of the *cis,trans*-germacradiene (1). Another plausible suggestion for biogenesis of 1α-hydroxy-*trans*-eudesmanolides is shown in Scheme 1; here the stereochemistry of the epoxide (4) does not dispose it towards acid-catalysed cyclization, but towards elimination. The allylic alcohol (5) is subsequently cyclized by the acid-catalysed process indicated (arrows) to one or all of the eudesmanolides represented by structure (7).[3]

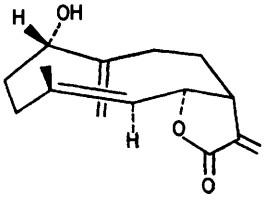


SCHEME 1

As far as we know, neither of the theories related to the biogenesis of 1α-hydroxy-*trans*-eudesmanolides has received experimental support. The object of this paper is to provide experimental evidence for the melampolide route proposed by Parker, Roberts, and Ramage.

Germacranolides with 1α-hydroxy-groups are not known, although Geissman *et al.*[4] originally suggested structure (8) for artemorin, and on the basis of ^{1}H n.m.r. analysis, assigned to it the conformation (14). Later





(14)

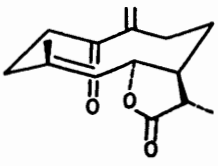
(8) R = OH, R′ = X = Y = H
(9) R = X = Y = H, R′ = OH
(10) R = OH, R′ = X = H, Y = Me
(11) R = OAc, R′ = X = H, Y = Me
(12) R = X = H, R′ = OH, Y = Me
(13) RR = O, X = H, Y = Me

El-Feraly *et al.*[5] reassigned the structure of artemorin as (9).

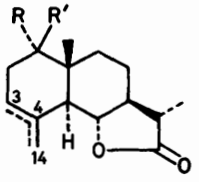
The absolute configuration and conformation of gallicin (12), a germacranolide isolated from *Artemisia maritima gallica*, have been determined in this laboratory.[6,7] The oxidation of (12) with MnO_2 followed by reduction with $NaBH_4$ gave 1-*epi*-gallicin (10). The stereoselectivity of the reaction may be due to the fact that the reduction takes place through a preferred reacting conformation (15).† The *si*-face of the carbonyl

† Unpublished results.

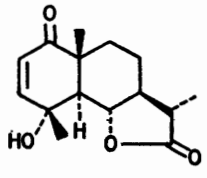
group at C-1 is highly hindered and attack by hydride ion takes place on the *re*-face.



(15)



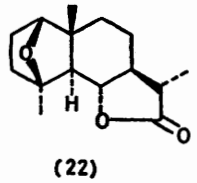
(16) R = OH, R′ = H, Δ^3
(17) R = OH, R′ = H, $\Delta^{4(14)}$
(18) RR′ = O, Δ^3
(19) R = H, R = OH, Δ^3
(20) R = H, R = OH, $\Delta^{4(14)}$



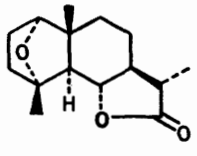
(21)

1-*epi*-Gallicin (10) undergoes a biogenetic-type cyclization to a mixture of eudesmanolides (16) and (17) when treated with protic acids. The *trans* stereochemistry of the *AB* ring junction was established by chemical transformations. Thus, oxidation of (16) with Jones reagent led to vulgarin (21), identical with an authentic sample isolated from *Artemisia canariensis* Lee.[8] Oxidation of (16) probably takes place *via* the ketone (18); indeed treatment of this ketone (obtained from vulgarin according to the method of Geissman *et al.*[9]) with Jones reagent led (in only 25% yield) to vulgarin (21).

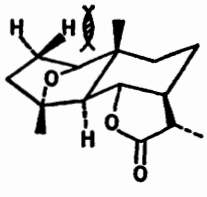
The cyclization to (16) and (17) is fully stereospecific; gallicin (12), by an identical process, leads to the *trans*-eudesmanolides (19), (20), and (22).[6] The absence of (23) in the cyclization products of (10) may be explained by steric interactions between H-2 and 10-Me [see (24)].



(22)



(23)



(24)

Under identical conditions, compounds (11) and (13) suffer no transformation, which suggests participation of the 3-OH *via* the protonated oxiran (26). A conformational study of (11) in solution was made by variable temperature ^{1}H n.m.r., with lanthanoid-induced shifts. The spectrum was taken at normal probe temperature (+35 °C), as none of the spectral features changed significantly between −60 and +60 °C. The addition of Eu(fod)$_3$ caused the shifts shown in the Figure. The

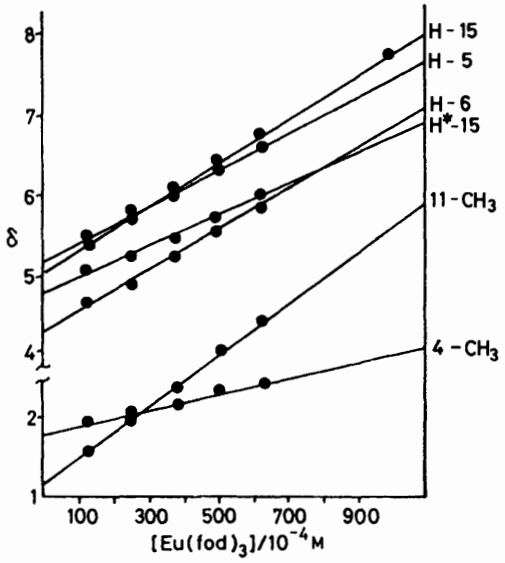
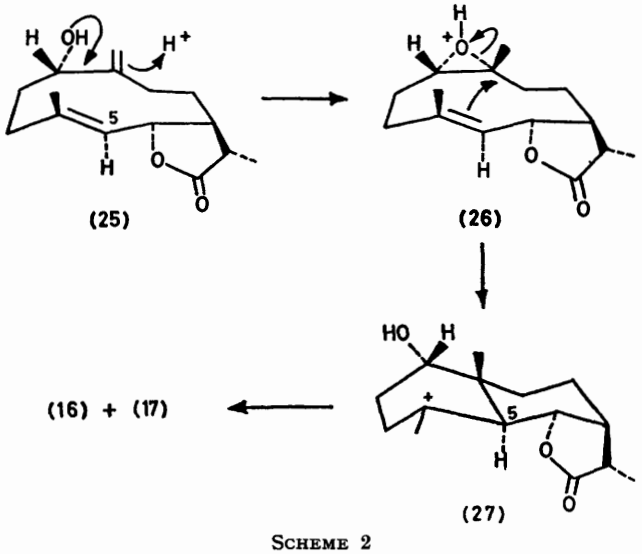


FIGURE Lanthanoid-induced shifts for compound (11)

shifts of the signals due to H-15 and H*-15 are the most important, which suggests the proximity of the acetoxy-function and the 10,15-double bond. The slight shift of the H-5 signal is not compatible with a *syn*-relationship of the acetoxy-group and H-5, which suggests the equatorial disposition of the acetoxy-function. These results are supported by comparison of the signals of H-1 in gallicin (12) and gallicin acetate with those of 1-*epi*-gallicin (10) and 1-*epi*-gallicin acetate (11), respectively; these signals are identical, which is only possible if the 1-OH is equatorial in both products (10) and (11).



(25) (26) (27)

(16) + (17)

SCHEME 2

These data agree with conformation (25) for 1-*epi*-gallicin and its acetate derivative (Scheme 2). Thus the transformation of 1-*epi*-gallicin (10) into 1α-hydroxy-*trans*-(10β,5α)-eudesmanolides occurs with the assistance of the 1α-OH, producing the intermediate oxiran cation (26) which can cyclise without difficulty (Scheme 2).

A Dreiding model of the *cis*-1(10),*trans*-4(5)-germacradienolides (the so-called melampolides) shows that compounds (1) are possible precursors of compounds of type (26). The conformation of (1) deduced for the melampolides from n.m.r. measurements and *X*-ray analysis [10] is exactly that required for cyclization to the naturally occurring 1α-hydroxy-*trans*-eudesmanolides. Moreover, none of the melampolides isolated [11] so far is a likely candidate for acid-catalysed cyclizations as above, because the carbonyl group at C-15 will deactivate the double bond.

EXPERIMENTAL

M.p.s were determined with a Kofler hot-plate apparatus. I.r. spectra were taken for solutions in $CHCl_3$, u.v. spectra for solutions in EtOH, and 90 MHz ^1H n.m.r. spectra for solutions in $CDCl_3$ (tetramethylsilane as internal reference). Optical rotations were measured for solutions in $CHCl_3$. Unless otherwise stated, column chromatography was carried out with Merck silica gel (0.05—0.2 mm).

Oxidation of Gallicin (12).—Active MnO_2 (freshly prepared; 3.5 g) was added to a solution of gallicin (250 mg) in CH_2Cl_2 (10 ml) and the mixture was stirred for 24 h. It was then filtered through Celite and concentrated *in vacuo*. Recrystallization from EtOAc–hexane gave the 1-*oxo-derivative* (15) (92%) of gallicin, m.p. 128—130°; $[\alpha]_D$ +169.6° (*c* 0.39); $\nu_{max.}$ 1 765 (γ-lactone) and 1 670 cm^{-1} (α,β-unsaturated ketone); *m/z* 248 (M^+); δ_H 1.24 (d, *J* 7 Hz, 11-Me), 1.75 (d, *J* 2 Hz, 4-Me), 4.35 (dd, *J* 10 and 9 Hz, 6-H), 5.02 (d, *J* 10 Hz, 5-H), and 5.66 and 5.82 (s, 15-H) (Found: C, 72.3; H, 7.85. $C_{15}H_{20}O_3$ requires C, 72.55; H, 8.1%).

1-epi-*Gallicin* (10).—The oxo-derivative (15) (172 mg) was dissolved in EtOH (10 ml) and cooled to 0 °C, and $NaBH_4$ (10 mg) was added. After 15 min the mixture was neutralized with dilute HOAc, extracted with EtOAc, dried ($MgSO_4$), concentrated *in vacuo* and chromatographed on silica gel with hexane–EtOAc (3 : 7) giving compound (10) (70%), m.p. 93—95° (from acetone–hexane); $[\alpha]_D$ +51.8° (*c* 0.21); $\nu_{max.}$ 3 612 (OH), 1 770 (γ-lactone), and 1 640 cm^{-1} (double bond); *m/z* 250.1564 and 232 (M^+ − 18); δ_H 1.22 (d, *J* 7 Hz, 11-Me), 1.72 (d, *J* 2 Hz, 4-Me), 3.98 (complex 1-H), 4.40 (dd, *J* 10 and 9 Hz, 6-H), 4.85 and 5.13 (s, 15-H), and 5.21 (d, *J* 9 Hz, 5-H).

Cyclization of 1-epi-*Gallicin* (10).—1-*epi*-Gallicin (10) (98 mg) was dissolved in CH_2Cl_2 (10 ml) and $CHCl_3$ (2 ml), through which HCl gas had been bubbled for 1 min, was added. The mixture was stirred at room temperature for 20 min, diluted with $CHCl_3$, washed with $NaHCO_3$ (1%), then H_2O, dried ($MgSO_4$), concentrated *in vacuo*, and chromatographed on silica gel impregnated with $AgNO_3$ (12%) yielding two products (16) and (17).

1α-*Hydroxy*-11βH-*eudesm*-3-*en*-12,6α-*olactone* (16) (35%) was recrystallized from acetone–hexane; m.p. 137—139° {lit.,[12] m.p. 130—131.5°; $[\alpha]_D$ +139°}, $[\alpha]_D$ +114.3° (*c* 0.21); $\nu_{max.}$ 3 615 (OH), 1 775 (γ-lactone), and 1 640 cm^{-1} (double bond); *m/z* 250 (M^+) and 232 (M^+ − 18); δ_H 0.86 (s, 10-Me), 1.21 (d, *J* 7 Hz, 11-Me), 1.86 (s, 4-Me), 3.29 (complex, 1-H), 3.99 (dd, *J* 10 and 9 Hz, 6-H), and 5.31 (br, s, 3-H) (Found: C, 71.75; H, 9.05. $C_{15}H_{22}O_3$ requires C, 71.95; H, 8.85%).

1α-Hydroxy-11βH-eudesm-4(14)-en-12,6α-olactone (17) (18%) could not be crystallized; $\nu_{max.}$ 3 620 (OH), 1 775 (γ-lactone), and 1 665 and 1 610 cm (double bond); *m/z* 250 (M^+) and 232 (M^+ − 18); δ_H 0.84 (s, 10-Me), 1.21 (d, *J* 7 Hz, 11-Me), 3.45 (complex, 1-H), 4.04 (dd, *J* 10 and 9 Hz, 6-H), and 4.79 and 4.94 (s, H-14).

Oxidation of the Lactone (16).—A solution of (16) (40 mg) in acetone (5 ml) was oxidized with Jones reagent at room temperature for 4 h. The solution was poured into cold water and extracted with $CHCl_3$; the extract was washed with aq. $NaHCO_3$ and water, dried, and evaporated to dryness. Chromatography on silica gel with EtOAc–light petroleum (1 : 1) yielded vulgarin (21) (11 mg, 27%), m.p. 176—177 °C, $[\alpha]_D$ +39° (*c* 0.3); $\nu_{max.}$ 3 520 (OH), 1 780 (γ-lactone), and 1 675 cm^{-1} (α,β-unsaturated ketone); $\lambda_{max.}$ 215 nm (EtOH); *m/z* 264 (M^+) and 246 (M^+ − 18); δ_H 1.23 (s, 10-Me), 1.28 (d, *J* 7 Hz, 11-Me), 1.55 (s, 4-Me), 2.36 (d, *J* 10 Hz, 5-H), 4.25 (dd, *J* 9 and 10 Hz, 6-H), 5.90 (d, *J* 10 Hz, 2-H), and 6.61 (d, *J* 10 Hz, 3-H) (Found: C, 68.35; H, 7.75. Calc. for $C_{15}H_{20}O_4$: C, 68.15; H, 7.5%).[8,9]

Deoxyvulgarin (18).—Vulgarin (21) (270 mg) was dissolved in boiling glacial acetic acid (5 ml) and zinc powder (675 mg) was added in small quantities during 45 min. The mixture was poured into water and extracted with EtOAc; the extract was washed with saturated aq. $NaHCO_3$, dried ($MgSO_4$), concentrated *in vacuo*, and chromatographed on silica gel with light petroleum–EtOAc (6 : 4) yielding deoxyvulgarin (18) (81%), which crystallized from acetone–hexane; m.p. 136—138°, $[\alpha]_D$ +72.6° (*c* 1.2); $\nu_{max.}$ 1 775 (γ-lactone), 1 715 (C=O), and 1 600 cm^{-1} (C=C); *m/z* 248 (M^+); δ_H 1.12 (s, 10-Me), 1.23 (d, *J* 7 Hz, 11-Me), 1.94 (br, s, 4-Me), 4.02 (t, *J* 9 Hz, 6-H), and 5.60 (br, s, 3-H).[9]

Oxidation of Deoxyvulgarin (18).—A solution of deoxyvulgarin (18) (150 mg) in acetone (15 ml) was oxidized at room temperature for 4 h with Jones reagent until an orange colour persisted. The solution was diluted with EtOAc, washed with water, dried, and evaporated to dryness, yielding 40 mg (25%) of vulgarin (21).

Acetate of 1-epi-*Gallicin* (11).—1-*epi*-Gallicin (10) (50 mg) when treated with acetic anhydride–pyridine formed the *acetate* (11) which crystallized from acetone–hexane; m.p. 121—123 °C, $[\alpha]_D$ −30.2° (*c* 0.4); $\nu_{max.}$ 1 780 (γ-lactone), 1 730 (acetate), 1 680, and 1 655 cm^{-1} (C=C); *m/z* 292 (M^+) and 250 (M^+ − 42); δ_H 1.25 (d, *J* 7 Hz, 11-Me), 1.80 (d, *J* 2 Hz, 4-Me), 2.04 (s, OAc), 4.44 (dd, *J* 10 and 9 Hz, 6-H), 4.86 and 5.19 (s, 15-H), 5.05 (complex, 1-H), and 5.24 (d, *J* 9 Hz, 5-H) (Found: C, 69.75; H, 8.75. $C_{17}H_{24}O_4$ requires C, 69.85; H, 8.45%).

This work was supported by the Assessorial Commission for Scientific and Technical Research of the Ministry of Universities and Investigation.

[1/624 *Received*, 22*nd April*, 1981]

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