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CHAPARROLIDE AND CASTELANOLIDE, NEW BITTER PRINCIPLES FROM *CASTELA NICHOLSONI*

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Abstract—Three additional lactones, two of them new, have been isolated from extracts of *Castela nicholsoni* Hook. from which chaparrin, glaucarubol, glaucarubol 15-isovalerate and amarolide have previously been obtained. The structures and stereochemistry of the new lactones have been established. Chaparrinone has also been found in the plant.

INTRODUCTION

THE DETAILED examination of *Castela nicholsoni* (Simaroubaceae) has been continued in the search for constituents that may possess the anti-amebic activity for which the plant is used in native medicine.[1] Since the major constituents, chaparrin and glaucarubol, showed no significant anti-amebic efficacy,[2] continuing studies have been directed to a search for new principles in which the putative activity may be found to lie.

Earlier studies on *C. nicholsoni* ('chaparro amargoso') have revealed the presence of chaparrin (I),[3] glaucarubol (II) and its 15-isovalerate (III),[4] glaucarubolone (IV)[5] and four lactones designated as compounds A (VI), D (VII), E (V) and F (VIII). Compound A has now been shown to be identical with amarolide[6] and a revised structure has been established.[7] Compound E had properties which suggested its identity as chaparrinone, and this was confirmed by comparison of physical data of E and its triacetate with that previously published for chaparrinone and chaparrinone triacetate.[5] The structures and stereochemistry of compounds D and F, named respectively chapparolide and castelanolide, are described in this paper.

* Contribution No. 2585 from the Department of Chemistry, UCLA.

[1] M. MARTINEZ, *Las Polantas Medicinales de Mexico*, p. 99, Ediciones Botas, Mexico; 3rd Ed. (1944).

[2] N. ENTNER, New York University School of Medicine; private communication. We are indebted to Dr. Entner for performing the biological assays on chaparrin and its derivatives.

[3] (a) T. A. GEISSMAN and K. R. CHANDORKAR, *J. Org. Chem.* **26**, 1217 (1961). (b) T. A. GEISSMAN and G. A. ELLESTAD, *Tetrahedron Letters* 1083 (1962). (c) P. de MAYO and T. A. DAVIDSON, *Can. J. Chem.* **43**, 2996 (1965).

[4] W. STÖCKLIN and T. A. GEISSMAN, *Phytochem.* In press.

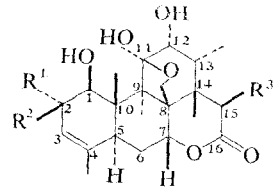
[5] J. POLONSKY and N. BOURGUIGNON-ZYLBER, *Bull. Soc. chim. France* 2793 (1965).

[6] C. G. CASINOVI, V. BELLAVITA, G. GRANDOLINI and P. CECCHERELLI, *Tetrahedron Letters* 2273 (1965).

[7] W. STÖCKLIN, C. G. CASINOVI, M. STEFANOVIĆ and T. A. GEISSMMAN, *Tetrahedron Letters* 2399 (1970).

RESULTS AND DISCUSSION

The compounds mentioned above are formulated as follows:



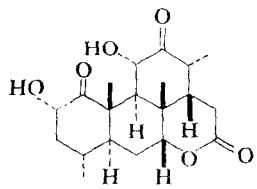
chapparin (I): $R^1 = OH$; $R^2 = R^3 = H$

glaucarubol (II): $R^1 = R^3 = OH$; $R^2 = H$

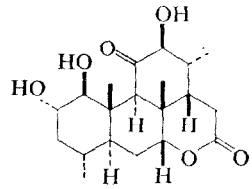
glaucarubol 15-isovalerate (III): $R^1 = OH$; $R^3 = OCOCH_2CH(CH_3)_2$; $R^2 = H$

glaucarubolone (IV): $R^1, R^2 = O$; $R^3 = OH$

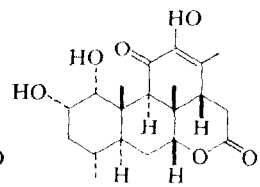
chaparrinone (V): $R^1, R^2 = O$; $R^3 = H$



Compound A (VI)
(amarolide)



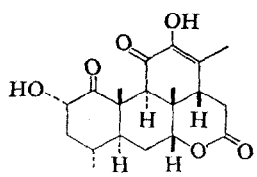
Compound D (VII)
(chaparrolide)



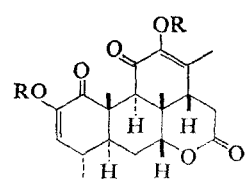
Compound F (VIII)
(castelanolide)

Compounds A, D, E and F were isolated from aqueous extracts of washings and mother liquors left after the isolation of chaparrin from *C. nicholsoni*. Extraction with chloroform yielded mixtures from which the individual compounds were separated by chromatography on silica gel columns. Compounds A, D, E and F are bitter but, in contrast to chaparrin and glaucarubol, give no color with concentrated sulfuric acid.[3]

Compound D (chaparrolide) had m.p. 125–130° and gave analytical figures agreeing with the constitution $C_{20}H_{30}O_6.2H_2O$. The mass spectrum showed the molecular ion at *m/e* 366, and the i.r. spectrum showed the presence of hydroxyl and carbonyl groups, the latter at 1725 and 1710 cm^{-1}, indicating the presence of a δ-lactone and a ketone grouping. The compound formed a triacetate (NMR and mass spectrum), m.p. 216–217°, showing the presence of three hydroxyl groups in chaparrolide and accounting for all of the oxygen atoms in the molecule. The NMR spectrum of chaparrolide was very similar in many respects to that of amarolide. In particular, it showed signals for two tertiary (δ 1·09 and 1·03) and two secondary (δ 0·93, $J = 5$ Hz; δ 1·17, $J = 6{\cdot}5$ Hz) methyl groups. The lactonic proton (C-7) appeared at δ 4·33.



IX



X, R = H

XI, R = CH_3

The surmise that chaparrolide was a glycol related to the diketone amarolide was substantiated by their oxidation with chromic acid to the same mixture of the diosphenol IX and the bis-diosphenol X. Both diosphenols gave positive ferric chloride tests. Compounds IX and X were characterized by their NMR, i.r. and mass spectra. In particular, each showed a vinylic (C-13) methyl group (δ 1·90); and while IX showed a one-proton quartet at δ 4·86 (J = 11·5, 7·5 Hz) for the β-hydrogen atom at C-2, compound X showed a vinylic proton doublet (δ 5·73, J = 2 Hz) for the C-3 proton. The structure of X was established by converting it into quassin (XI)[8] by methylation.

That the carbonly function of chaparrolide was indeed in ring C was established by its oxidation in acetic acid with bismuth trioxide to a monoacetate of the C-ring diosphenol.* The oxidation product gave no color with ferric chloride. It showed a molecular ion (*m/e* 406) in the mass spectrometer, and prominent fragment ions at 391 (M-15), 388 (M-18), 364 (M-42), 346 (M-42-18) and 331 (M-42-18-15). Its NMR spectrum showed signals for a vinylic methyl group (3 H, δ 1·88) and an acetyl methyl group (3 H, δ 2·08), in addition to the appropriate signals for two tertiary and one secondary methyl groups and for the C-7 and C-9 hydrogen atoms. In summary, all of the evidence leads to the assignment of structure VII to chaparrolide (compound D). An alternate structure, in which the positions of the carbonyl and hydroxyl groups in ring C are reversed, was discarded on the following evidence, which permits assignment of the complete structure.

The 100 MHz spectrum of chaparrolide triacetate showed, in addition to seven 3-proton signals for the methyl groups and a triplet-like signal for the C-7 (lactonic) proton, a doublet for the proton at C-1 (δ 4·67; J = 10 Hz), coupled with the C-2 proton, which appeared as a sextet (due to further coupling with CH_2 at C-3) centered at δ 4·98 (J = 10, 10, 5 Hz). The C-1/C-2 protons are thus *trans*-diaxially disposed. Signals for two protons between δ 2·65 and 2·85 contained a singlet for the C-9 proton at δ 2·72, showing that the carbonyl group is at C-11. A doublet for the C-12 proton of C<u>H</u>OAc (δ 4·85, J = 11·5 Hz) coupled with the proton of C<u>H</u>CH_3 at C-13 (which appeared as a multiplet at δ 2·38) establishes a *trans*-diaxial relationship of the C-12/C-13 protons.

These assignments were confirmed by double resonance experiments. Irradiation of the doublet at δ 4·85 (C-12) produced decoupling only of the multiplet at δ 2·38 (C-13), which remained a multiplet due to coupling with CH_3 and the proton at C-14. Irradiation at δ 2·38 reduced the signal for the C-12 proton to a singlet. In contrast, irradiation of the singlet at δ 2·76 (C-9) caused no change in the rest of the spectrum. These observations, coupled with the conversion of chaparrolide into quassin, are all in accord with VII, in respect both to the positions of the functional groups and to the stereochemistry.

Compound F (castelanolide) had m.p. 165–167° and the molecular ion at *m/e* 364 in its mass spectrum indicated the composition $C_{20}H_{28}O_6$. The i.r. spectrum showed absorption for hydroxyl groups and for the carbonly groups of a lactone (1730 cm^{-1}) and a diosphenol (1690 and 1660 cm^{-1}). The compound gave a color with ferric chloride and its u.v. spectrum showed a maximum at 276 nm (log ε = 3·79), characteristic of a diosphenol.

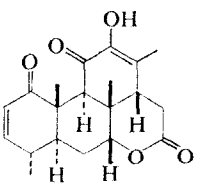
The NMR spectrum of castelanolide showed three-proton signals for two tertiary methyl groups (δ 1·13 and 1·26), one secondary methyl group (δ 1·10; J = 1·5 Hz) and one vinylic methyl group (δ 1·88); a one-proton singlet for the C-9 proton (δ 2·82); and a triplet for the

* The formation of the acetate of the diosphenol in the oxidation of chaparrol has been observed by P. de Mayo and T. A. Davidson.[3c]

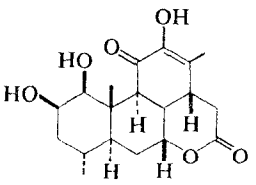
[8] Z. Valenta, A. H. Gray, D. E. Orr, S. Papadopoulos and C. Podseva, *Tetrahedron* **18**, 1433 (1962).

lactonic proton at C-7 (δ 4·30; $J = 2$ Hz). These observations suggested that castelanolide possessed a structure closely allied to that of amarolide, and the absence of vinylic protons indicated that the disosphenol was in ring C. That the compound was a 1,2-*cis*-diol was indicated by the appearance of a doublet at δ 4·56 with $J = 3$ Hz, which was shifted to δ 5·93 on acetylation.

Support for these structural assignments was afforded by oxidation of castelanolide with chromic acid to give a mixture from which the diosphenol IX and the bis-diosphenol X could be isolated. Direct comparison of IX and X from this experiment with the materials prepared earlier from chaparrolide established their identity. A third, minor, component of the reaction mixture resulting from the oxidation of castelanolide was assigned structure XII on the basis of its i.r., u.v. and mass spectrum, and the fact that it showed the diosphenol color reaction with ferric chloride. These findings permitted the formulation of castelanolide as VIII or XIII. Structure VIII was regarded as more acceptable than XIII in view of the formation, upon oxidation, of compound IX in which the C-2 hydroxyl group is α-disposed. This conclusion was substantiated by decoupling experiments. The 100 MHz spectrum of castelanolide triacetate showed, in addition to the signals for seven methyl groups, signals for protons at C-1 (doublet, δ 5·89; $J = 3$ Hz), C-2 (multiplet, δ about 5·24), C-7 (multiplet, δ 4·33) and C-9 (singlet, δ 2·84). Irradiation of the doublet at δ 5·89 caused the multiplet at δ 5·24 to change to a quartet ($J = 11·5$, 5 Hz), representing the C-2 proton now coupled only with CH_2 at C-3. Irradiation of a multiplet at δ 1·66 (CH_2 at C-3) caused the δ 5·24 multiplet to collapse to a doublet ($J = 3$ Hz) (C-2 proton now coupled only to C-1). Simultaneous irradiation at 5·89 and 1·66 ppm caused the multiplet at δ 5·24 (C-2) to become nearly a singlet. These observations substantiate the structure and stereochemistry as shown in VIII.



XII



XIII

Reaction of castelanolide with acetic anhydride-pyridine at 5° for 30 min yielded a monoacetate, which, because of the lack of a ferric reaction, must be the enol acetate of the diosphenol (only the OH at C-12 acetylated), and which was, as expected, different from the bismuth trioxide oxidation product of chaparrolide.

EXPERIMENTAL

M.ps were determined in capillaries on a Büchi melting point apparatus and are corrected. I.r. spectra were taken as nujol mulls unless otherwise specified. NMR spectra were recorded on Varian Associates A-60-A, A-60-D, T-60 and HA-100 instruments, double resonance experiments on the latter, and peak positions are given in δ values with tetramethylsilane as an internal standard; mass spectra were obtained on an AEI-MS-9 spectrometer, with a direct inlet, at 70 eV ionization potential, and significant ions at higher *m/e* values as well as ions with a relative abundance of 30% or more of the base peak are quoted as *m/e* values, with their relative abundance in parenthesis. TLC was on precoated Merck F_{254} plates (5 × 10 cm or 5 × 20 cm when the adsorbate was particularly complex), elution being achieved with benzene–ethyl formate (1:1) or $CHCl_3$–methanol (97:3, 17:3), visualization being satisfactory after spraying with conc. H_2SO_4 and heating in an oven at 120° for a few minutes; column chromatograms used Baker silica gel 60–200 mesh

(30 g/l. g of adsorbate) packed in $CHCl_3$, and were eluted with increasing portions of acetone in $CHCl_3$, their progress being monitored by TLC. Analytical samples were dried at 80°/0·1 mm for 24 hr immediately prior to analysis.

Fractionation of the extracts of Castela nicholsoni. Crude extracts remaining after separation of chaparrin,[3a] which were bitter and gave a purple color with conc. H_2SO_4,* were combined, the organic solvents evaporated and the remaining aqueous solution concentrated and extracted successively with pentane, $CHCl_3$, $CHCl_3$–alcohol (3:2). The pentane extract showed no bitterness and was discarded. The $CHCl_3$ and $CHCl_3$–alcohol extracts were each concentrated to dryness; the residue from the latter yielded only chaparrin and glaucarubol as crystalline material, while the residue from the former was found, on close examination, to contain additional crystalline compounds, *viz.*, compounds A, E and F, and D, all very bitter to the taste but giving no color to conc. H_2SO_4, and isolated from fractions eluted from a column chromatogram with $CHCl_3$–acetone 98:2 and 95:5, 90:10 and 70:30 (E and F mixture) and acetone, respectively.

Compound E, chaparrionone (V), was obtained by rechromatographing fractions yielding F, by eluting with $CHCl_3$–acetone (92:8) and recrystallization from $CHCl_3$ as colorless needles, m.p. 245–248° (lit.[5] 238–242°), *m/e* 378·1678 (M^+) (92), calculated for $C_{20}H_{26}O_7$: 378·1678. *Anal*: found: C, 63·43; H, 7·07; calcd. for $C_{20}H_{26}O_7$: C. 63·68; H, 6·93%. Compound E formed a triacetate m.p. 138–143° (lit.[5] 135–137°). The i.r. and NMR spectral data for compound E and its triacetate were practically identical with the same spectral data previously published.[5]

Compound A, amarolide (VI), is described elsewhere.[6] Its oxidation is described here, however, because of its correlation with compounds D and F.

Oxidation of Amarolide (VI); Compounds IX and X

(i) *With chromic anhydride.* To a solution of amarolide (VI) (250 mg. 0·67, mM) in HOAc (5 ml) was added a 10% (w/v) solution (1 ml, 1·01 mM) of CrO_3 in 10% (v/v) aq. HOAc and the solution heated on a steam bath for 3 hr, then evaporated. The residue was slurried with $CHCl_3$ filtered, (celite), and the $CHCl_3$ evaporated to give a green residue which recrystallized from ethanol to yield colorless needles consisting of two components (TLC) which did not separate on further recrystallizations. Chromatography on a column (39 × 1·1 cm) of silica gel (12 g) by eluting with $CHCl_3$ gave good separation of the product.

Compound X, eluted first from the column, was obtained as colorless needles after recrystallization of appropriate fractions from ethanol; dark green $FeCl_3$ color; m.p. 187–192° (lit.[6] 207–208°); ν_{max} 3400, 1735 (shoulder), 1710 (shoulder), 1690, 1665, 1280, 1235, 1200 and 1050 cm^{-1}; δ ($CDCl_3$) 1·08 (3 H, d; J = 6·5 Hz), 1·18 (3 H, s), 1·50 (3 H, s), 1·86 (3 H, s), 2·42 (1 H, s), 4·30 (1 H, t; $J \approx 2$ Hz) and 5·73 (1 H, d; $J \approx 2$ Hz); *m/e* 360 (M^+) (14), 345 (M^+-15) (16), 91 (50), 77 (40), 69 (80), 55 (34), 53 (38), 43 (100) and 41 (93).

Compound IX, eluted immediately after X from the column and recrystallized as colorless prisms from 95% ethanol; m.p. 256·263° (dec); ν_{max} 3460, 3405, 1735, 1715, 1690, 1660, 1280, 1240, 1200, 1065 and 960 cm^{-1}; δ ($CDCl_3$) 0·94 (3 H, d; J = 6 Hz); 1·23 (3 H, s), 1·44 (3 H, s), 1·90 (3 H, s), 2·47 (1 Hs, s), 3·36 (1 H, s), 4·29 (1 H, t; J = 2·5 Hz) and 4·86 (1 H, q; J = 11·5 and 7·5 Hz); *m/e* 362 (M^+) (28), 347 (M^+-15) (4), 91 (47), 79 (30), 77 (31), 55 (50), 53 (36), 43 (82) and 41 (100). *Anal.* found: C, 66·51; H, 7·16; calc. for $C_{20}H_{26}O_6$: C, 66·28; H, 7·23%.

(ii) *With bismuth trioxide.* Amarolide (VI) (250 mg, 0·69 mM) in HOAc (10 ml) was stirred with bismuth trioxide (256 mg, 0·55 mM) for 5 days at 50° or 22 hr at reflux. In each case the reaction mixture was evaporated to dryness and the products isolated by column chromatography as above. The oxidation at 50° yielded mainly compound IX, m.p. 260–264° (dec), and the latter oxidation (at reflux) mainly compound X, m.p. 208–210°, each having i.r. spectra indistinguishable from those of IX and X previously isolated.

Methylation of Compound X; Quassin (XI)

Attempted methylations of compound X (50 mg, 0·14 mM) with CH_2N_2 were not successful, so the evaporated reaction mixture in a solution of acetone (10 ml) containing Me_2SO_4 (38 mg, 0·28 mM) was refluxed and stirred with anhydrous K_2CO_3 for 4 hr. TLC examination of the reaction after its filtration and evaporation indicated it to be ca. 50% completed, and after the addition of further acetone (5 ml), Me_2SO_4 (35 mg), and K_2CO_3 (40 mg), the reaction was completed with further refluxing for 6 hr. The reaction mixture was filtered, evaporated, taken up in $CHCl_3$ and placed on a column (1 × 18 cm) of silica gel (5 g) prepared in $CHCl_3$. The main product, giving a TLC spot identical with that of quassin, was eluted by $CHCl_3$, and crystallized from cold ether; recrystallization from 2-propanol then afforded colorless needles, m.p. 215–218°; mixed m.p. 218–219·5° with authentic quassin, m.p. 221–2°; and i.r. spectrum identical with that of quassin. *Anal.*: found: C, 67·66; H, 7·45; calc. for $C_{22}H_{28}O_6$, C, 68·02; H, 7·27%.

Compound D, chaparrolide (VII), m.p. 125–130° after recrystallization from ethanol or acetone; ν_{max} 3450–3250, 1735, 1275, 1090 and 1025 cm^{-1}; δ (pyridine-d_5) 0·72 (3 H, d; J = 5·5 Hz), 1·10 (3 H, s), 1·19

* A color reaction for many of the simaroubzceous bitter principles, characteristic for the allylic alcohol in ring-A.

(3 H, d; J = 6·5 Hz), 1·51 (3 H, s), 3·01 (1 H, s) (C-9 H), and other unanalyzed complex signals; *m/e* 366 (M^+) (12), 351 (M^+-15) (2), 348 (M^+-18) (21), 333 (M^+-18-15) (8), 330 (M^+-18-18) (5), 119 (40), 107 (47), 105 (34), 95 (32), 93 (36), 91 (41), 81 (30), 79 (33), 69 (30), 67 (40), 55 (88), 43 (90) and 41 (100). *Anal.*: found: C, 59·82; H, 8·46; calc. for $C_{20}H_{30}O_6 \cdot 2H_2O$: C, 59·82; H, 8·46%.

Oxidation of Chaparrolide (VII); Correlation of VII with IX and X

(i) *With chromic anhydride.* A solution of chaparrolide (VII) (200 mg, 0·546 mM) in HOAc (4 ml) containing 1·5 ml (*ca.* 1·46 mM) of a 10% solution of CrO_3 in 10% aq. HOAc was heated on a steam bath for 2·5 hr and worked up in the usual way. The crude acetone-soluble product was chromatographed as above, and led to the isolation of two crystalline (from ethanol) products. The first, m.p. 190–194°, had an R_f (TLC) identical to and an i.r. spectrum superimposable on that of compound X while the second, m.p. 253–258·5° (dec), had R_f identical to and i.r. spectrum superimposable on that of compound IX.

(ii) *With bismuth trioxide.* Chaparrolide (VII) (100 mg, 0·27 mM) and bismuth trioxide (140 mg, 0·30 mM) were stirred with HOAc (4 ml) in an oil bath held at 80° for 3 days, then cooled and evaporated. The residue was slurried with a small volume of saturated aq. $KHCO_3$ then evaporated and the resulting salts extracted with acetone. The oil remaining after evaporation of the acetone was chromatographed in the usual manner to yield a main product which crystallized from ether–benzene to give the 12-monoacetate of the ring-C diosphenol from VII as a colorless solid, m.p. 163–166°; ν_{max} 3300 (broad), 1740 (str), 1685 (med), 1640 (str), 1250 and 1035 cm^{-1}; δ ($CDCl_3$) 0·89 (3 H, d; J = 5·5 Hz), 1·21 (6 H, s), 1·88 (3 H, s), 2·08 (3 H, s), 2·72 (1 H, s) and 4·26 (1 H, broad but apparently a triplet; *m/e* 406 (M^+) (2), 364 (M^+-42) (2), 346 (M^+-42-18) (35), 331 (M^+-42-18-15) (8), 328 (M^+-42-18-18) (2) and 43 (100). *Anal.*: found: C, 63·40; H, 7·25; calc. for $C_{22}H_{30}O_7 \cdot 1/2\ H_2O$, C, 63·63; H, 7·52%.

Acetylation of Chaparrolide (VII). A solution of chaparrolide (VII) (150 mg) in pyridine (1 ml) containing Ac_2O (0·3 ml) was kept at room temp overnight and then poured into ice cold 1N HCl (10 ml). The aq. acid solution was extracted with $CHCl_3$ (5 × 7 ml) and the combined extracts dried, filtered, evaporated and the residue crystallized from a small volume of 2-propanol. The colorless crystalline material so obtained was recrystallized from 2-propanol, affording colorless needles; m.p. 216–217°; ν_{max} 1740, 1280 (sh), 1270 (str), 1220 (sh), 1075, 1065, 1050 and 1040 cm^{-1}, δ ($CDCl_2$) 0·90 (3 H, d; J = 6 Hz), 1·01 (3 H, d; J = 6·5 Hz), 1·12 (3 H, s), 1·43 (3 H, s), 1·84, 1·95, 2·12 (3 × 3 H, s), 2·38 (mult), 2·72 (1 H, s), 2·76 (1 H, d (?); J = 12 Hz ?), 4·29 (1 H, t; J = 3 Hz), 4·67 (1 H, d; J = 10 Hz), 4·85 (1 H, d; J = 11·5 Hz) and 4·98 (1 H, sextet; J = 10, 10, 5 Hz); *m/e* 492 (M^+) (4), 450 (M^+-42) (10), 432 (M^+-42-18) (13), 408 (M^+-2 × 42) (18), 390 (M^+-2 × 42-18) (100), 375 (390-15) (25), 372 (390-18) (23) and 330 (37·5). *Anal.*: found: C, 63·56; H, 7·60; calc. for $C_{26}H_{39}O_9$; C, 63·40; H, 7·37%.

Compound F, castelanolide (VIII), had m.p. 167° after recrystallization from $CHCl_3$; ν_{max} 3500, 3400–3340, 1730, 1685, 1655, 1235 and 1040 cm^{-1}; λ_{max} 276 nm (log ϵ = 3·79); δ (acetone-d_6) 1·10 (3 H, d), 1·13 (3 H, s), 1·26 (3 H, s), 1·88 (3 H, s), 2·82 (1 H, s), 4·39 (1 H, *t*-like) and 4·56 (1 H, d; J = 3 Hz); *m/e* 364 (M^+) (5), 346 (M^+-18) (100), 331 (M^+-18-15) (18), 328 (M^+-2 × 18) (2), 313 (M^+-2 × 18-15) (12), 209 (40), 43 (37) and 41 (38). *Anal.*: found: C, 64·25; H, 7·52; calcd. for $C_{20}H_{28}O_6 \cdot 1/2\ H_2O$, C, 64·36; H, 7·83%.

Oxidation of Castelanolide (VIII); Correlation of VIII with IX and X; Compound XII

A solution of castelanolide (VIII) (300 mg, 0·83 mM) in HOAc (6 ml) was oxidized in the usual manner with 1·15 ml of a 10% CrO_3 in 10% aq. HOAc. TLC showed the reaction product to be very complex; column chromatography as before led to the isolation of compound IX, m.p. 254–260° (dec) after recrystallization from ethanol, with i.r. spectrum superimposable on that of IX previously isolated. Rechromatography of three other fractions, combined, led to the isolation of a small quantity of compound X with i.r. spectrum superimposable on X previously isolated.

The first fraction from the column contained a compound of higher R_f (TLC) than X; it crystallized from a cold ether solution of the fraction, m.p. 239–294° (dec). It gave a green $FeCl_3$ color. ν_{max} 3455 (—OH), 3045 (HC=CH), 1740 (α-lactone C=O), 1675 (sh), 1670, 1665 (sh), (α,β-unsaturated ketone and diosphenol) and 1585 (C=C) cm^{-1}; λ_{max} 234 (log ϵ = 4·17) and 275 nm (log ϵ = 3·54); *m/e* 344 (M^+) (100), 258(40), 91 (42), 43 (40) and 41 (42).

Acetylation of Castelanolide (VIII)

(i) *Triacetate.* Castelanolide (VIII), when acetylated in the normal manner, yielded a TLC-homogeneous but amorphous glass which could not be induced to crystallize; ν_{max} 1740, 1690, 1665 (w), 1250 and 1035 cm^{-1}; δ ($CDCl_3$) 0·96 (3 H, d), 1·25 and 1·32 (2 × 3 H, s), 1·66 (mult), 1·82 (3 H, s), 1·92, 2·02, 2·20 (3 × 3 H, s), 2·82 (1 H, s), 4·30 (1 H, mult), 5·24 (1 H, mult) and 5·89 (1 H, d; J = 3 Hz); *m/e* 490 (M^+) (0·15), 448 (M^+-42) (14), 330 (M^+-42-18) (2), 388 (M^+-42-18-42) (89), and 43 (100). The compound was not analyzed.

(ii) *Monoacetate.* To castelanolide (VIII) (50 mg) in a solution of pyridine (1 ml) cooled to 5° was added Ac_2O (0·15 ml) and after 30 min the reaction was poured into ice-cold 1N HCl and extracted with $CHCl_3$

(4 × 5 ml). The combined extracts were washed with saturated aq. $KHCO_3$, dried and, evaporated to yield a crude product showing two components on TLC and no castelanolide. The same reaction was incomplete during 30 min at 0°. The lower-R_f product, isolated by way of column chromatography followed by recrystallization from ether-acetone, had m.p. 152–158°; ν_{max} 3500–3400, 1750 (sh), 1740, 1690, 1670 (sh), 1225, 1125 and 1040 cm^{-1}; δ ($CDCl_3$) 0·88 (3 H, broad, secondary methyl). 1·05 (3 H, s), 1·30 (3 H, s) (two tertiary methyls), 1·83 (3 H, s) (vinylic methyl), 2·24 (3 H, s) (acetyl methyl), 4·00 (1 H, broad mult) (C-2 H), 4·30 (1 H, mult) (C-7 H) and 4·49 (1 H, d; J = 2·5 Hz) (C-1 H); *m/e* 406 (M^+) (1·4), 388 (M^+-18) (49), 209 (61), 165 (32), 123 (35), 105 (30), 91 (48), 79 (32), 69 (33), 67 (33), 55 (68) 53 (30), 44 (48), 43 (100), 42 (35) and 41 (83). *Anal.*: found: C, 63·11; H, 7·53; calc. for $C_{22}H_{30}O_7 \cdot 1/2\ H_2O$, C, 63·63; H, 7·52%.

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