Minor Diterpenoid Constituents of *Andrographis paniculata* Nees

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Three diterpenoids closely related to andrographolide have been isolated from *Andrographis paniculata* Nees. These have been identified as 14-deoxy-11-oxoandrographolide (6), 14-deoxy-11,12-didehydroandrographolide (23), and 14-deoxyandrographolide (26).

THE tissue cultures of *Andrographis paniculata* have been found [1,2] to produce the sesquiterpenoid lactones, paniculides A (1), B (2), and C (3), and apparently lack the ability of the whole plant to synthesise diterpenoids. Only the diterpenoids andrographolide (4) [3] and neoandrographolide (5) [4] have been isolated from the whole plant. It was of interest, therefore, to reinvestigate the whole plant to determine whether the foregoing sesquiterpenoids, or related compounds, were present among the minor constituents. Extraction of *A. paniculata* afforded, in addition to andrographolide (4) and neoandrographolide (5), three new crystalline natural products † closely related to andrographolide. No sesquiterpenoids were detected.

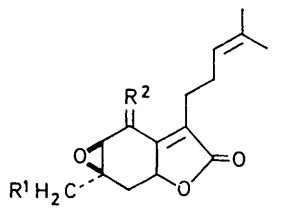
The first compound, $C_{20}H_{28}O_5$, m.p. 98—100°, $[\alpha]_D$ −13°, was shown to be 14-deoxy-11-oxoandrographolide (6). The functional groups were readily revealed spectroscopically. Thus the compound had hydroxy-groups [$\nu_{max.}$ (CCl_4) 3608 and 3500 cm^{-1}], an αβ-unsaturated γ-lactone system [$\nu_{max.}$ (CCl_4) 1766 cm^{-1}; $\lambda_{max.}$ 227 nm (ε 9000)], a ketonic carbonyl [$\nu_{max.}$ (CCl_4) 1721 cm^{-1}], and an exocyclic methylene group [$\nu_{max.}$ (CCl_4) 1647 and 902 cm^{-1}]. The n.m.r. spectrum showed signals for two tertiary methyl groups, one primary and one secondary hydroxy-group [δ 3·72 (ABq, J_{AB} 12 Hz) and 3·50 (m)], and an exocyclic methylene [δ 4·43 and 4·84 (both s)], and two mutually coupled doublets [δ 7·51 (1H) and 4·85 (2H), J 1·8 Hz] consistent [5] with the presence of an endocyclic double bond in conjugation with the γ-lactone carbonyl. A second AB quartet centred at δ 3·43 (J 18 Hz) was subsequently ascribed to the C-12 methylene protons.

The positional and stereochemical assignment of the two hydroxy-functions of (6) rests on the following evidence. First, the n.m.r. spectra of andrographolide (4) and its acetate (7) exhibit characteristics extremely similar to those of (6) and the corresponding diacetate (8). This, together with the co-occurrence of the compounds suggests that they possess the same part structure (9). Secondly, it is known that the methylene protons of C-4 acetoxymethyl groups resonate at lower field (δ *ca.* 4·3) when the function has an axial rather than an equatorial orientation.[6] The corresponding protons in compound (8) appear as an AB quartet centred at δ 4·26, consistent with the presence of an axial CH_2·OAc system. Finally, treatment of (6) with benzaldehyde and zinc chloride gave the benzylidene derivative

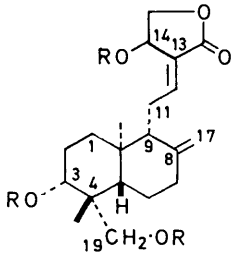
† 14-Deoxy-11,12-didehydroandrographolide and 14-deoxyandrographolide have been described previously as transformation products of andrographolide but not as natural products.

[1] A. J. Allison, D. N. Butcher, J. D. Connolly, and K. H. Overton, *Chem. Comm.*, 1968, 1493.
[2] D. N. Butcher and J. D. Connolly, *J. Exp. Botany*, 1971, **22**, 314.
[3] M. P. Cava, W. R. Chan, R. P. Stein, and C. R. Willis, *Tetrahedron*, 1965, **21**, 2617.
[4] W. R. Chan, D. R. Taylor, C. R. Willis, R. L. Bodden, and H.-W. Fehlhaber, *Tetrahedron*, 1971, **27**, 5081.
[5] M. P. Cava, B. Weinstein, W. R. Chan, J. L. Haynes, and L. F. Johnston, *Chem. and Ind.*, 1963, 167.
[6] E. Wenkert and P. Beak, *Tetrahedron Letters*, 1961, 358.

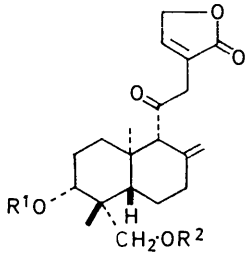
(10). Consequently the two hydroxy-groups must have a *cis*-relationship, with the C-3 OH also α.



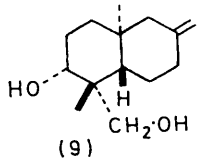
(1) $R^1 = H, R^2 = H$, β-OH
(2) $R^1 = OH, R^2 = H$, β-OH
(3) $R^1 = OH, R^2 = O$



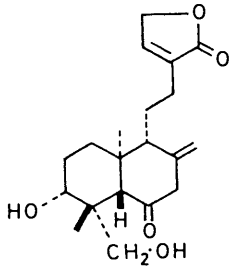
(4) R = H
(7) R = Ac



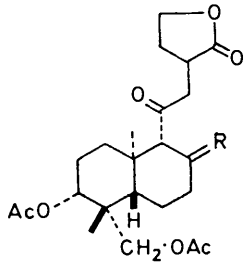
(6) $R^1 = R^2 = H$
(8) $R^1 = R^2 = Ac$
(10) $R^1R^2 = PhCH$



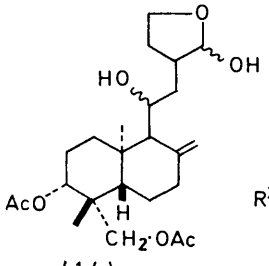
(9)



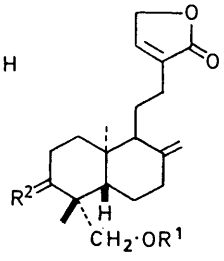
(11)



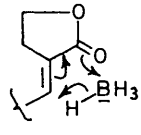
(12) R = O
(13) $R = CH_2$



(14)



(5) R^1 = β-D-glucosyl, $R^2 = H_2$
(15) $R^1 = Ac, R^2 = H$, α-OAc
(26) $R^1 = H, R^2 = H$, α-OH



(16)

The foregoing evidence eliminates all but two possible structures, (6) and (11). A decision in favour of (6) was reached by a more detailed analysis of the n.m.r. and mass spectra and confirmed chemically by the formation of the β-diketone (12) (see later).

In the n.m.r. spectra of both compounds (6) and (8) the olefinic proton (H-14) resonates as a doublet (J 1·8 Hz), δ *ca.* 7·50. The other compounds in this series which contain an endocyclic αβ-unsaturated γ-lactone system have the equivalent resonance within the range δ 7·2—7·02. This deshielding of >0·3 p.p.m. can be accounted for by placing the ketonic carbonyl group at C-11, where it is suitably disposed to influence the chemical shift of H-14. In the alternative formulation (11) the C-6 carbonyl group is too far removed to have any appreciable deshielding effect. In addition, double-resonance experiments on the diacetate (8) demonstrate a small allylic coupling between H-14 and the C-12 methylene protons, which resonate as an AB system at δ 3·44.

Reduction of the diacetate (8) with sodium borohydride in aqueous methanol afforded a 1 : 1 mixture of products, assigned the structures (13) and (14), respectively. The less polar was the keto-lactone (13) [$\nu_{max.}$ (CCl_4) 1783, 1742, and 1720 cm^{-1}]. The n.m.r. spectrum confirmed the loss of the characteristic butenolide signals. The more polar product was the hydroxy-lactol (14) [$\nu_{max.}$ ($CHCl_3$) 3610, 3440, and 1730 cm^{-1}], which formed a tetra-acetate. Oxidation with Jones reagent transformed the hydroxy-lactol into the keto-lactone (13).

The formation of compounds (13) and (14) as the only isolable products from this borohydride reduction raises some points of mechanistic interest. Normally the exocyclic double bond of an αβ-unsaturated butenolide is reduced by borohydride.[7,8] On the other hand, the endocyclic butenolide system is unaffected under normal conditions.[3] 14-Deoxyandrographolide diacetate (15), formed by borohydride reduction of andrographolide triacetate (7), is not further reduced under the conditions of its formation.[3] The mechanism postulated to rationalise these observations is one involving a cyclic intermediate (16) [7] favoured by the cisoid unsaturated carbonyl system.

In view of this stability of endocyclic butenolide double bonds to reduction with borohydride, the formation of compounds (13) and (14) must involve, as a first step, the isomerisation (8) ⟶ (17) (Scheme 1). Normal reduction can then proceed to give the enolate (18), which leads to the keto-lactone (13).

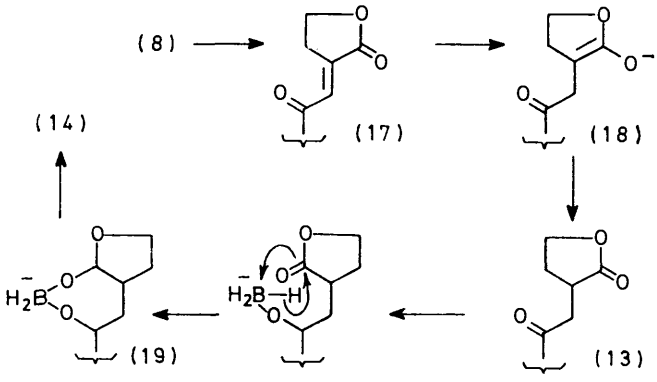
The formation of the hydroxy-lactone (14) requires protonation of the enolate (18) by the aqueous methanol medium. A slower reduction of the C-11 carbonyl group can then be followed by intramolecular hydride transfer to the lactone carbonyl. This furnishes the cyclic intermediate (19) which, on acidic hydrolysis, leads to the observed hydroxy-lactol (14). This mechanism rationalises the failure to isolate either the hydroxy-lactone (20) or the keto-lactol (21) from the reaction.

Hydroxylation of the keto-lactone (13) with osmium tetroxide in benzene afforded the corresponding diol,

[7] C. Djerassi and W. Rittel, *J. Amer. Chem. Soc.*, 1957, **79**, 3528.

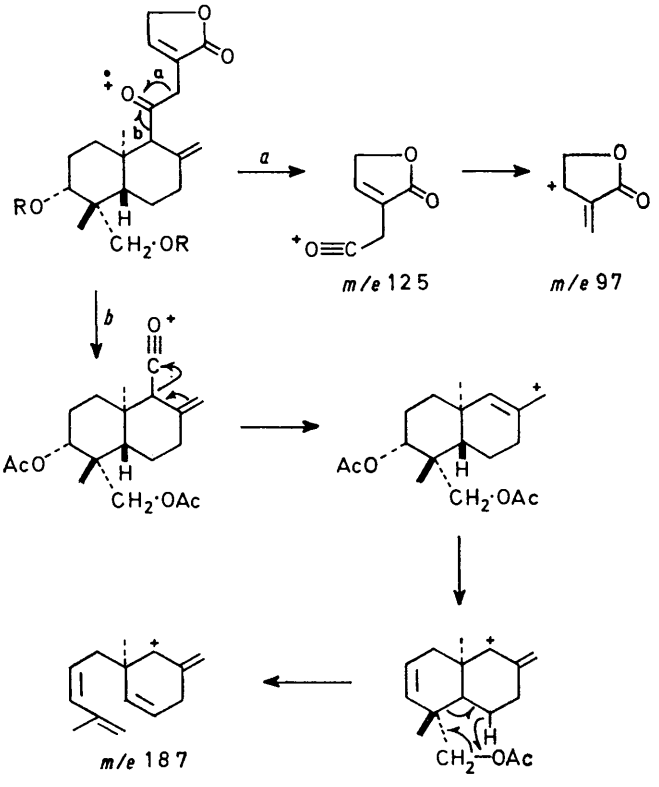
[8] M. M. Mehra, K. G. Deshpanda, B. B. Ghatge, and S. C. Bhattacharya, *Tetrahedron*, 1967, **23**, 2469.

which was cleaved with sodium periodate to give the β-diketone (12) [$\nu_{max.}$ (CCl_4) 1778, 1743, and 1712 cm^{-1};



SCHEME 1

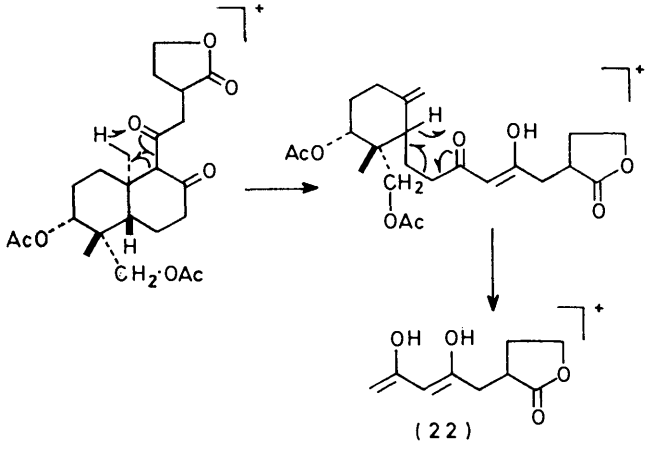
$\lambda_{max.}$ (with one drop of 0·1 M-NaOH) 309 nm (ε 7500)]. In keeping with its 'flexible' nature[9] the β-diketone existed wholly in the diketo-form in neutral ethanol.



SCHEME 2

This result confirms the placing of the carbonyl group at C-11.

The mass spectra of 14-deoxy-11-oxoandrographolide (6) and its derivatives provided additional structural evidence. The primary fragmentation is the ready α-cleavage initiated[10] by the C-11 ketonic carbonyl group (see Scheme 2). This gives rise to the base peak at *m/e* 125 in the case of the benzylidene derivative (10) [*m/e* 127 in the β-diketone 12)]. This ion can lose carbon monoxide to form the fragment at *m/e* 97, a transition which is supported by a metastable peak at *m/e* 75·3. A prominent peak at *m/e* 187 in the spectrum of the diacetate (8) probably arises by the alternative α-cleavage of the 11,12-bond followed by elimination of carbon monoxide and acetic acid (Scheme 2). In addition, the β-diketone (12) undergoes a ready double McLafferty rearrangement leading to the ion (22) (Found: *m/e*, 184·0734. $C_9H_{12}O_4$ requires 184·0735) (see Scheme 3).



SCHEME 3

The other two natural products were of similar polarity and were eventually separated by chromatography on silver nitrate-impregnated plates. The major compound, $C_{20}H_{28}O_4$, m.p. 203—204°, had strong u.v. absorption [$\lambda_{max.}$ 248 nm (ε 11,000)]. The n.m.r. spectrum revealed a typical bicyclic nucleus with two tertiary methyl groups, one primary and one secondary hydroxy-group, an exocyclic methylene, and the familiar endocyclic butenolide. In addition it indicated the presence of a *trans*-disubstituted double bond [δ 6·72 (q, *J* 16 and 10 Hz) and 5·93 (d, *J* 16 Hz)]. The appropriate decoupling experiments suggested that this compound was 14-deoxy-11,12-didehydroandrographolide (23).

Acetylation of andrographolide has been reported[3] to proceed with elimination to give the deoxydidehydroandrographolide diacetate (24). In our hands, acetylation under normal conditions afforded mainly the enol lactone (25) [$\nu_{max.}$ ($CHCl_3$) 1780 cm^{-1}].* However acetylation under reflux gave only the diacetate (24), identical with the diacetate of the natural product (23).

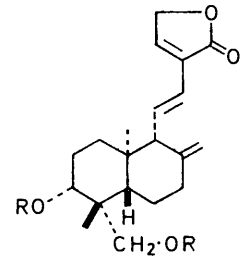
The third compound, $C_{20}H_{30}O_4$, m.p. 174—175°, displayed features in the n.m.r. spectrum similar to those

* This enol lactone has not been previously described. Presumably more forcing conditions used by other workers have resulted in isomerisation of the initially formed (25) to give the thermodynamically more stable diacetate (24).

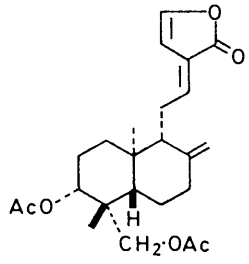
[9] A. I. Scott, 'Interpretation of the Ultraviolet Spectra of Natural Products,' Pergamon, Oxford, 1964, p. 69.

[10] J. H. Beynon, R. A. Saunders, and A. E. Williams, 'Mass Spectra of Organic Molecules,' Elsevier, Amsterdam, 1968, p. 190.

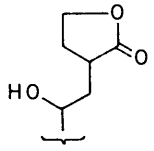
of its congener (23), with the exception of the signals for the disubstituted double bond. This suggested that the compound was 14-deoxyandrographolide (26). Authentic 14-deoxyandrographolide diacetate (15) was



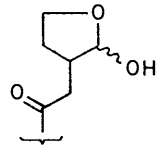
(23) R = H
(24) R = Ac



(25)



(20)



(21)

prepared [3] by borohydride reduction of andrographolide triacetate, and was identical with the diacetate of the natural product.

EXPERIMENTAL

N.m.r. spectra were obtained with a Varian T-60 or HA 100 spectrometer. Mass spectra were determined with an A.E.I. MS12 spectrometer, high resolution spectra being obtained with an A.E.I. MS902S instrument. Rotations were run for solutions in chloroform.

The dry powdered whole plant of *A. paniculata* (1 Kg) was extracted (Soxhlet) with ethyl acetate. The total extract was chromatographed on neutral alumina (grade IV). Gradient elution was performed with benzene changing to chloroform and then to 20% methanol–chloroform. The fractions were monitored by t.l.c. and rechromatographed on preparative plates to give the following compounds. Andrographolide (4) (6 g) crystallised from ethanol as plates, m.p. 230—231° (lit.,[3] 227·5°). *14-Deoxy-11-oxoandrographolide* (6) (1·2 g) was obtained as needles, m.p. 98—100° (from chloroform–ether), $[\alpha]_D$ −13°, $\lambda_{max.}$ 227 nm (ε 9000), $\nu_{max.}$ (CCl_4) 3608, 3500, 1766, 1720, 1642, and 902 cm^{-1}, δ 0·98 and 1·20 (tertiary methyls), 3·43 (ABq, *J* 18 Hz, 12-H_2), 3·50 (m, 3-H), 3·72 (ABq, *J* 12 Hz, 19-H_2), 4·43 and 4·84 (each s, 17-H_2), 4·85 (d, *J* 1·8 Hz, 15-H_2), and 7·51 (d, *J* 1·8 Hz, 14-H) (Found: C, 68·65; H, 8·3. $C_{20}H_{28}O_5$ requires C, 68·95; H, 8·1%). 14-Deoxy-11,12-didehydroandrographolide (23) and 14-deoxyandrographolide (26) were separated by repeated chromatography on silver nitrate-impregnated preparative plates. *14-Deoxy-11,12-didehydroandrographolide* (23) (600 mg) crystallised from ether as fine needles, m.p. 203—204°, $\nu_{max.}$ ($CHCl_3$) 3608, 3500, 1758, 1643, and 880 cm^{-1} (Found: C, 72·5; H, 8·65. $C_{20}H_{28}O_4$ requires C, 72·25; H, 8·5%).

[11] M. P. Cava, W. R. Chan, K. L. Haynes, L. F. Johnston, and B. Weinstein, *Tetrahedron*, 1962, **18**, 397.

14-Deoxyandrographolide (26) (200 mg) crystallised from ether–light petroleum as needles, m.p. 175° (lit.,[3,11] 170—171°) $\nu_{max.}$ ($CHCl_3$) 3608, 3500, 1759, 1645, and 902 cm^{-1} (Found: C, 71·9; H, 9·0. $C_{20}H_{30}O_4$ requires C, 71·8; H, 9·05%).

Neoandrographolide (5) (50 mg), m.p. 167—168° (lit.,[4] 167—168°) was isolated in low yield from the methanolic extract of *A. paniculata* by column chromatography on silica gel. It was characterised as the tetra-acetate, m.p. 156—157° (lit.,[4] 155—157°) (Found: C, 62·85; H, 7·4. Calc. for $C_{34}H_{48}O_{12}$: C, 62·95; H, 7·45%).

Diacetate (24) *of Natural 14-Deoxy-11,12-didehydroandrographolide* (23).—Compound (23) (20 mg) was acetylated with acetic anhydride (0·5 ml) and pyridine (0·5 ml) at 20° overnight. Crystallisation from chloroform–ether afforded the diacetate (24) (21 mg) as needles, m.p. 134—135° (lit.,[3] 136—137°), $\lambda_{max.}$ 246 nm (ε 13,000), identical with an authentic sample (Found: C, 69·25; H, 7·8. Calc. for $C_{24}H_{32}O_6$: C, 69·2; H, 7·75%).

Diacetate (15) *of Natural 14-Deoxyandrographolide* (26).—Compound (26) (15 mg) similarly gave the diacetate (15) (15 mg), which crystallised from ether as needles, m.p. 118—120° (lit.,[12] 120°), $\lambda_{max.}$ 220 (ε 8500) and 230 nm (4400), identical with an authentic sample (Found: C, 69·0; H, 8·3. Calc. for $C_{24}H_{34}O_6$: C, 68·9; H, 8·2%).

Acetylation of Andrographolide (4).—(*a*) A solution of andrographolide (190 mg) in pyridine (2 ml) and acetic anhydride (1 ml) was left at 20° for 16 h. Methanol was added and the solvents were removed *in vacuo* to give a gum which was shown by t.l.c. to contain two products. The less polar *enol lactone* (25) (126 mg) crystallised from chloroform–ether as needles, m.p. 139—142°, $\lambda_{max.}$ 299 (ε 7300) and 224 nm (7000), $\nu_{max.}$ ($CHCl_3$) 1780, 1730, 1648, and 900 cm^{-1}, δ 6·14 (d, *J* 4 Hz, 14-H), 6·68 (t, *J* 8 Hz,12-H), and 6·98br (d, $W_{\frac{1}{2}}$ 6 Hz, 15-H) (Found: C, 69·0; H, 7·85. $C_{24}H_{32}O_6$ requires C, 69·2; H, 7·75%). The more polar component was crystallised from chloroform–ether to give 14-deoxy-11,12-didehydroandrographolide diacetate (24) (40 mg) as fine needles, m.p. 134—135°.

(*b*) Andrographolide (60 mg) was refluxed in pyridine (2 ml) and acetic anhydride (1 ml) for 2½ h and the mixture was left at room temperature overnight. Analytical t.l.c. of the crude product showed only a trace of the enol lactone (25), the major component being 14-deoxy-11,12-didehydroandrographolide diacetate (24) (55 mg).

(*c*) Triacetylandrographolide (7), prepared [12] by heating andrographolide in acetic anhydride in the presence of freshly fused zinc chloride for 5 min, crystallised from ethanol as silky needles, m.p. 128—129° (lit.,[12] 128°).

Triacetylandrographolide (15 mg) was refluxed in dry pyridine (2 ml) overnight. The product was 14-deoxy-11,12-didehydroandrographolide diacetate (24) (12 mg), m.p. 134—135°.

14-Deoxyandrographolide Diacetate (15).—Reduction of triacetylandrographolide (55 mg) with sodium borohydride [3] afforded 14-deoxyandrographolide diacetate (15) (45 mg), which crystallised from ether as needles, m.p. 118—119° (lit.,[3] 120°), identical with the diacetate obtained by acetylation of natural 14-deoxyandrographolide (26).

Benzylidene Derivative (10) *of 14-Deoxy-11-oxoandrographolide* (6).—A solution of compound (6) (40 mg) in freshly distilled benzaldehyde (3 ml) was shaken for 14 h at 20° with freshly fused powdered zinc chloride (60 mg).

[12] D. Chakravarti and R. M. Chakravarti, *J. Chem. Soc.*, 1952, 1697.

The *benzylidene derivative* (10) (38 mg) was obtained as a gum, $\nu_{max.}$ (CCl_4) 1767 and 1720 cm^{-1}, which was sublimed at 180° and 0·02 mmHg (Found: C, 74·05; H, 7·4. $C_{27}H_{32}O_5$ requires C, 74·3; H, 7·4%).

14-Deoxy-11-oxoandrographolide Diacetate (8).—14-Deoxy-11-oxoandrographolide (6) (50 mg) was acetylated in the usual manner to afford the *diacetate* (8) (51 mg) as a gum, $\nu_{max.}$ (CCl_4) 1766 and 1740—1720 cm^{-1}, which was sublimed at 180° and 0·1 mmHg (Found: C, 66·05; H, 7·45. $C_{24}H_{32}O_7$ requires C, 66·65; H, 7·45%).

Sodium Borohydride Reduction of 14-Deoxy-11-oxoandrographolide Diacetate (8).—The diacetate (8) (91 mg) in aqueous methanol (1 : 4; 6 ml) was stirred for 30 min with an excess of sodium borohydride (65 mg). Analytical t.l.c. indicated the presence of two compounds in the crude product (84 mg). These were separated by preparative t.l.c. The less polar was the non-crystalline saturated *keto-lactone* (13) (60 mg), $\nu_{max.}$ (CCl_4) 1783, 1740, and 1720 cm^{-1} (Found: *m/e*, 434. $C_{24}H_{34}O_7$ requires *m/e*, 434). The minor product was the *hydroxy-lactol* (14) (20 mg), which crystallised from ether as needles, m.p. 120—123°, $\nu_{max.}$ (CCl_4), 3610, 3440, and 1730 cm^{-1} (Found: C, 65·5; H, 8·7. $C_{24}H_{38}O_7$ requires C, 65·75; H, 8·75%). Acetylation under normal conditions gave the corresponding non-crystalline *tetra-acetate* (Found: *m/e*, 522. $C_{28}H_{42}O_9$ requires *m/e*, 522).

Oxidation of the Hydroxy-lactol (14).—The hydroxy-lactol (14) (10 mg) in acetone (2 ml) was treated with Jones reagent (2 drops) at 0° for 15 min. The product, homogeneous by t.l.c., was identical (t.l.c., i.r., n.m.r.) with the keto-lactone (13).

The β-Diketone (12).—Osmium tetroxide (30 mg) in benzene (1 ml) was added dropwise to a solution of the keto-lactone (13) (54 mg) in benzene (3 ml) and pyridine (10 drops) and the mixture was left overnight at room temperature. The osmate was decomposed by treatment with hydrogen sulphide. The crude diol (50 mg) in methanol (3 ml) was treated with sodium periodate (40 mg) in water (2 ml) for 16 h. The product was purified by preparative t.l.c. to give the *β-diketone* (12) (36 mg) as a gum, $\nu_{max.}$ (CCl_4) 1778, 1743, and 1712 cm^{-1}, u.v. (EtOH) end absorption, $\lambda_{max.}$ (1 drop 0·1M-NaOH) 309 nm (ε 7500), which was sublimed at 190° and 0·2 mmHg (Found: *m/e*, 436·2095. $C_{23}H_{32}O_8$ requires *m/e*, 436·2097).

One of us (A. B.) acknowledges receipt of an S.R.C. Studentship.

[2/2591 *Received*, 15*th November*, 1972]