Tetrahedron. Vol 27, pp. 5081 to 5091. Pergamon Press 1971. Printed in Great Britain

THE STRUCTURE AND STEREOCHEMISTRY OF NEOANDROGRAPHOLIDE, A DITERPENE GLUCOSIDE FROM *ANDROGRAPHIS PANICULATA* NEES

W. R. CHAN, D. R. TAYLOR, C. R. WILLIS and R. L. BODDEN
Chemistry Department, University of the West Indies, Kingston 7, Jamaica

and

H.-W. FEHLHABER
Organisch-Chemisches Institut, Universität Bonn, West Germany

(*Received in USA* 9 *June* 1971; *Received in the UK for publication* 15 *June* 1971)

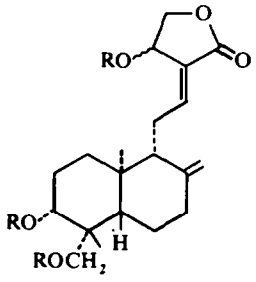
Abstract – Neoandrographolide (*2*) is shown to be the β-glucoside of *ent*-19-hydroxy-8(17), 13-labdadien-16,15-olide. A correlation with andrographolide has been done.

EXTRACTS of the shrub *Andrographis paniculata* Nees (Acanthaceae), common in the West Indies and India. are extensively used as household medicines in these areas.[1] The main constituent of *A. paniculata* is the diterpenoid lactone, andrographolide (**1**).[2] The isolation and characterisation of a second, minor, crystalline component. neoandrographolide. m.p. 167–168°. was described by Kleipool.[3] He suggested the molecular formula $C_{23}H_{38}O_8$ for neoandrographolide and from solubility experiments and a positive Legal test deduced the presence of an α,β-unsaturated γ-lactone. The preparation of an acetate. m.p. 157°. which was considered to be an anhydrotetraacetate. was also described. We now report our further investigations on neoandrographolide which lead to the constitution **2**.[4]

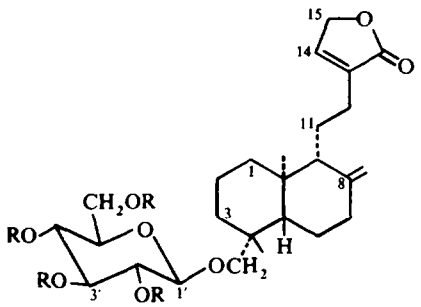
Elemental analysis of a number of derivatives combined with mass spectrometric results (see later) indicate a molecula formula $C_{26}H_{40}O_8$ for neoandrographolide. There was a single CO band (ν_{max} 1748 cm^{-1}) in the IR and this with UV data [λ_{max} 205 nm (ε 10,400)] supported the presence of an α,β-unsaturated butenolide already inferred by Kleipool. Four OH groups were indicated by strong IR absorption near 3300 cm^{-1} and by the formation of a tetraacetate (**3**), $C_{34}H_{48}O_{12}$, m.p. 155–157°. This acetate has a m.p. in good agreement with that reported by Kleipool for his anhydrotetraacetate but a direct comparison has not been possible. The acetate (**3**) has no OH absorption in the IR and can be reconverted to neoandrographolide. A medium intensity band at 909 cm^{-1} indicated the presence of an exocyclic methylene group. The presence of two ethylenic bonds in neoandrographolide (**2**) was confirmed by the formation of the tetrahydro derivative (**4**) on catalytic hydrogenation.

The NMR spectrum of the acetate (**3**) shows signals for four acetates and two tertiary Me groups. There is a narrow multiplet at δ 7·10 ($W\frac{1}{2}$ = 4 Hz) assigned to the β-proton of the α,β-unsaturated lactone system. The chemical shift is similar to that found in analogous systems[5] while the small coupling constant with the adjacent

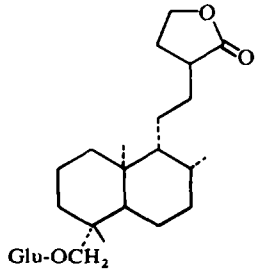
protons suggests that the double bond is endocyclic rather than exocyclic.[2] The spectrum also shows a complex of signals between δ 3·1 – 5·2 integrating for 13 protons which was not amenable to analysis.



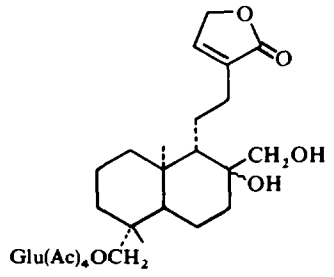
1: R = H
15: R = Ac



2: R = H
3: R = Ac



4



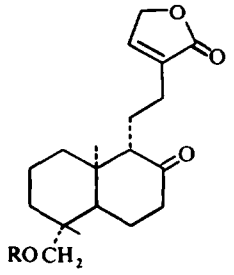
5

Reaction of the acetate (**3**) with osmium tetroxide in dioxan afforded the diol (**5**). $C_{34}H_{50}O_{14}$, m.p. 143–145°, which shows retention of the butenolide moiety in the UV. The formation of the diol (**5**) involved the exocyclic methylene and in agreement there are no bands associated with this group in the IR. Periodate cleavage of the diol gave, in good yield, formaldehyde (isolated as the 2,4-dinitrophenylhydrazone) and the norketone acetate (**6**). $C_{33}H_{46}O_{13}$, m.p. 145–147°. This compound may also be obtained directly from the acetate (**3**) by oxidation with the osmium tetroxide–sodium periodate reagent.[6] Hydrolysis of **6** afforded the tetrol **7**.

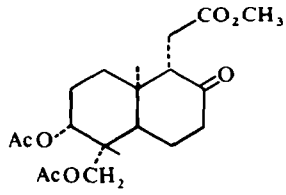
Spectral data for the norketone acetate (**6**) are in accord with the assigned structure. The butenolide system is associated with a maximum in the UV at 207 nm (ε 8950) while in the IR there is a broad CO band (1754–1739 cm^{-1}) for the acetate and lactone functions and another at 1701 cm^{-1} ascribed to a cyclohexanone. In the NMR spectrum H-14 appeared as a broadened singlet at δ 7·16 ($W\frac{1}{2}$ = 4 Hz).

The location of the ketone and the stereochemistry of the bicyclic system was established by CD data. The curve for **6** shows a positive Cotton effect [$\Delta\varepsilon_{max}$ + 2·74 at 293 nm, inflexions at 300 ($\Delta\varepsilon$ + 2·64) and 310 nm ($\Delta\varepsilon$ + 1·56)] and is virtually superimposable on that of the andrographolide derivative (**8**) [$\Delta\varepsilon_{max}$ + 2·67 at 289 nm, inflexions at 297 ($\Delta\varepsilon$ + 2·41) and 305 nm ($\Delta\varepsilon$ + 1·39)]. This result places the ketone at C(8) and indicates an identity in the relative and absolute configurations of the decalin systems.

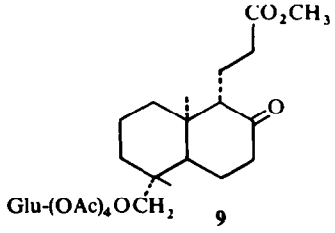
Oxidation of the tetraacetate (3) with the sodium periodate-potassium permanganate reagent[7] gave an amorphous acid which was characterized as the crystalline methyl ester, $C_{31}H_{46}O_{13}$, m.p. 142–144°, by treatment with diazomethane. Spectral data (Experimental) are in agreement with the structure 9.



6: R = Glu(Ac)$_4$
7: R = Glu

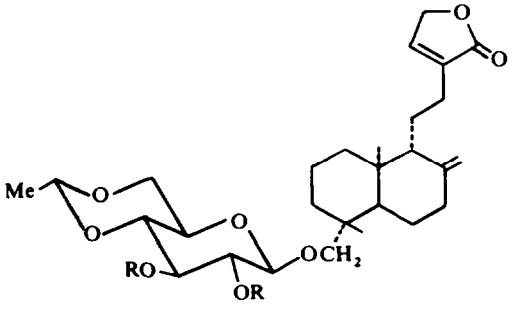


8

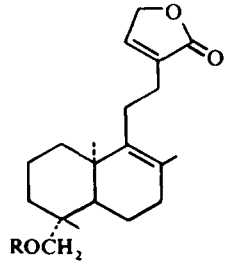


9

The presence of a glucose moiety in neoandrographolide was established as follows. In the iodometric estimation neoandrographolide required 1·58 moles of periodate after 48 hr. With acetaldehyde and zinc chloride, an acetylidene derivative (10) was obtained which was further characterized as the diacetate (11). Strong absorption in the IR near 890 cm^{-1} indicated that the exocyclic methylene is intact in these compounds. Treatment of neoandrographolide with hydrochloric acid in ethanol under reflux gave the iso-aglucone (12), $C_{20}H_{30}O_3$, m.p. 121–123°, together with glucose which was identified by two dimensional paper chromatography using appropriate hexoses as markers. The β-configuration of the glucoside was clearly shown by the NMR spectrum of the norketone acetate (6) in which H-1′ appears as a doublet (J = 7·5 Hz) at δ 4·45.

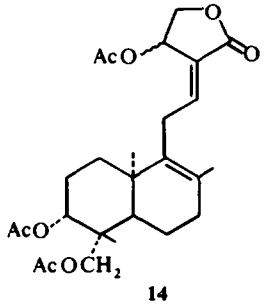


10: R = H
11: R = Ac

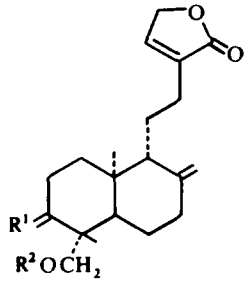


12: R = H
13: R = Ac

Acetylation of the iso-aglucone (**12**) gave the acetate (**13**), also obtained from **2** by treatment with acetic acid and conc sulphuric acid. The structure of the acetate (**13**) follows from its genesis and spectral data. The NMR spectrum shows 3 singlet methyls, one of which is vinylic, and one acetate. Above $\delta = 3$, there are a total of five protons in distinct spin systems. An AB quartet at δ 4·10 (J = 11·5 Hz, ν_{AB} 19·6 Hz) can be assigned to the acetoxymethyl group. H-15 appears as a doublet (J = 1·3 Hz) at δ 4·78 and H-14 as a narrow multiplet at δ 7·17. The chemical shift of the acetoxymethyl group is in good agreement with that found in methyl podocarpate and in andrographolide derivatives where this group is axial.[2]



14



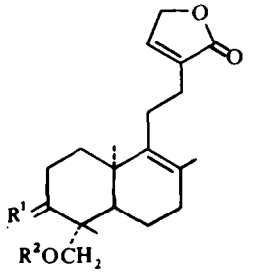
16: R^1 = H, α-OH; R^2 = H
17: R^1 = H, α-OH; R^2 = CPh_3
18: R^1 = O; R^2 = CPh_3
19: R^1 = SCH_2CH_2S; R^2 = H

$Ph_3CSCH_2CH_2SCPh_3$

20

$Ph_3CSCH_2CH_2SH$

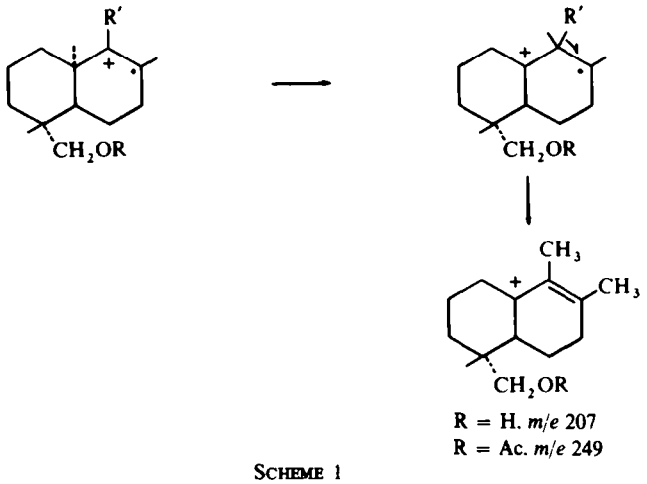
21



22: R^1 = O; R^2 = Ac
23: R^1 = $NNHSO_2C_6H_4Me$: R^2 = Ac

The base peak (*m/e* 287) in the mass spectrum of both **12** and **13** corresponds to the ion $C_{19}H_{27}O_2^+$ formed by loss of the CH_2OR group. Surprisingly, there are significant fragments which must be formed by elimination of the entire side-chain, i.e. by a homolytic cleavage of the C(9)–C(11) bond. Thus in **12**, there is a peak at *m/e* 207 (40%) and in **13**, peaks at *m/e* 249 (25%) and 189 (249-AcOH, 54%). The composition of all the ions mentioned was confirmed by precise mass measurements. Since direct cleavage of a vinylic bond is not usually a favoured process, it is possible this fragmentation is preceded by a rearrangement of the double bond to allow an allylic cleavage or, alternatively, a Me migration, as indicated in Scheme 1, is involved.

The double bond isomerization associated with the formation of the iso-aglucone under acidic conditions is not surprising. Indeed, andrographolide (**1**) gave the triacetate (**14**) under conditions identical for the conversion of **2** to **13**. In the NMR



SCHEME 1

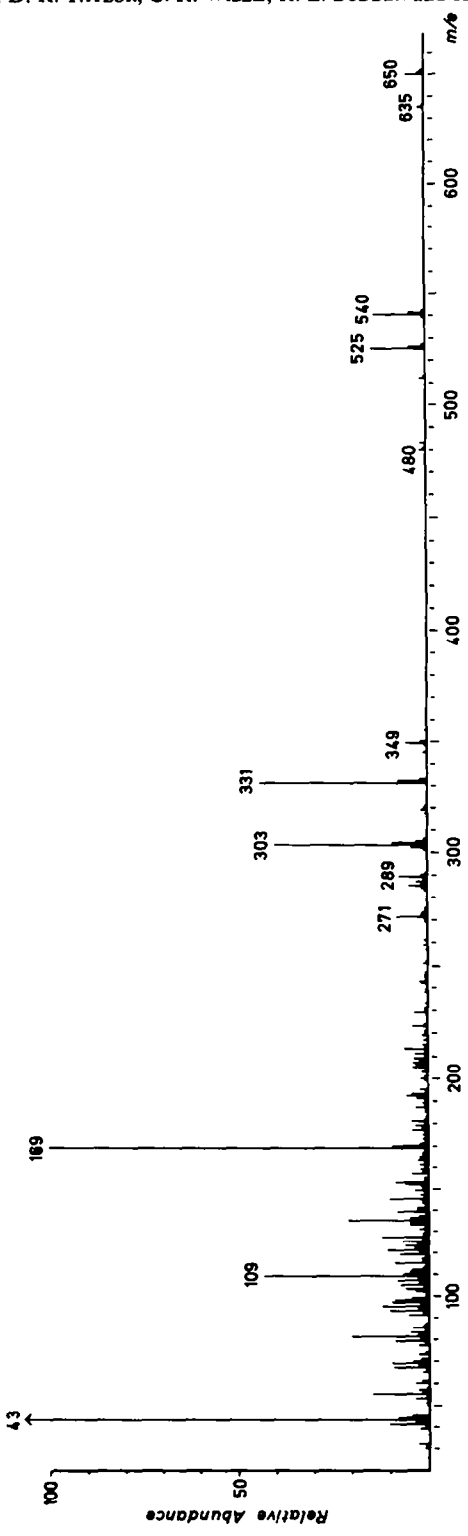
spectrum. this triacetate has two tertiary methyls, a vinylic methyl. H-14 as a diffuse doublet at δ 5·93 and H-12 as a triplet (J = 7 Hz) at 6·92 shown to be spin-coupled to the two protons at C (11) which appear as a diffuse doublet at 3·00. In the isomeric triacetate (**15**), H-12 occurs as a triplet at δ 6·97 (J = 6·5 Hz) and is coupled to the protons on C(11) at 2·43.

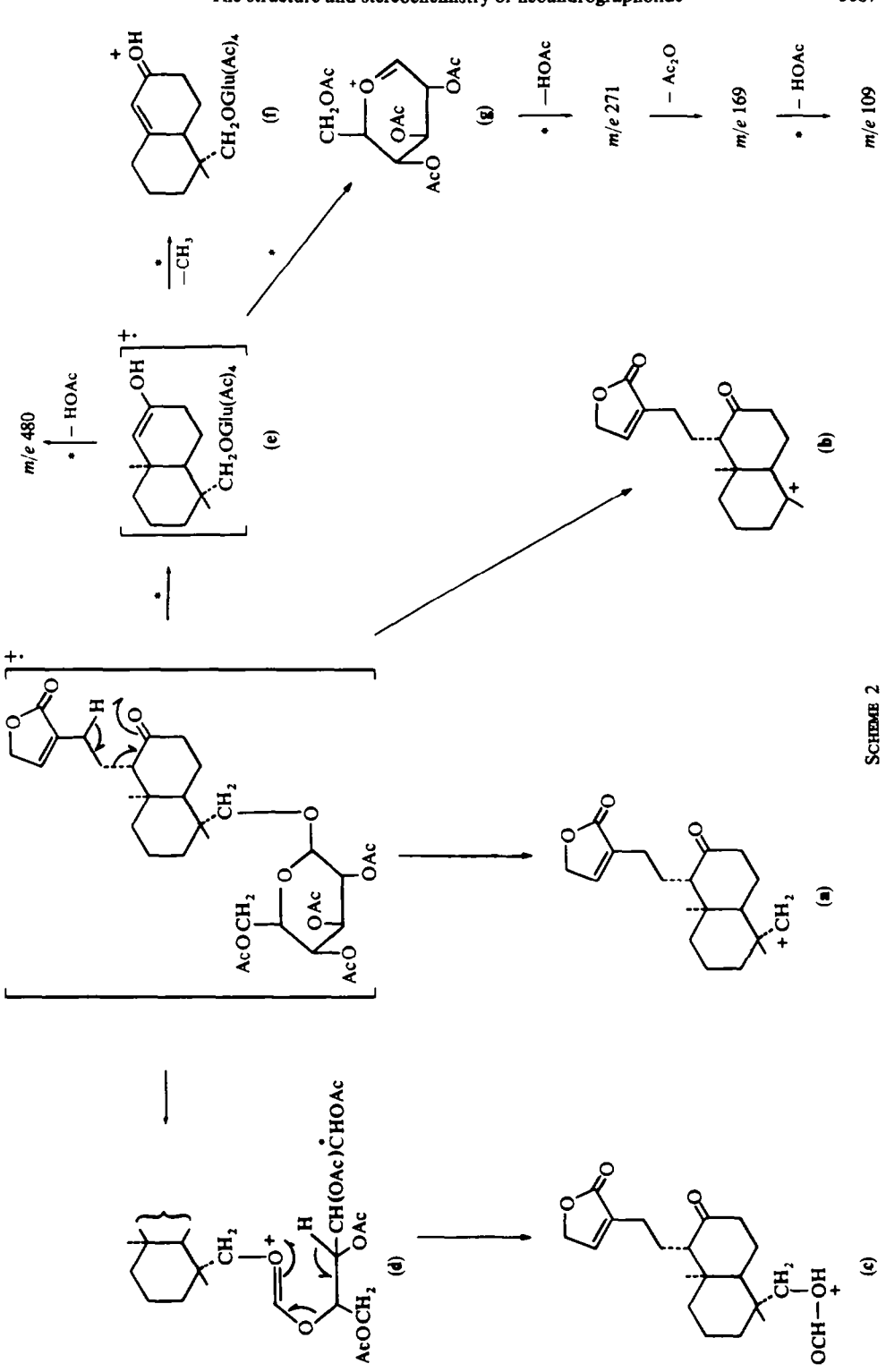
The high resolution mass spectrum of the norketone acetate (**6**) (Fig. 1) further delineates many of the features already established. For a sugar derivative it shows a surprisingly strong parent ion at *m/e* 650 (5%) confirming the molecular formula $C_{33}H_{46}O_{13}$. Three fragment ions at *m/e* 303, 289 and 349 are formed by loss of the sugar moiety. These correspond to the ions $C_{19}H_{27}O_3^+$ (**a**), $C_{18}H_{25}O_3^+$ (**b**) and $C_{20}H_{29}O_5^+$ (**c**) (scheme 2). The formation of (**c**) is initiated by cleavage of C(1')–C(2') in the sugar followed by a McLafferty rearrangement as indicated in (**d**). An analogous fragmentation is found in the steroidal sapogenins.[8]

Several metastable peaks indicate that the main fragmentation path proceeds through the ion (**e**) of *m/e* 540. This is obtained from the parent ion through a McLafferty rearrangement involving the C(8) CO group. Subsequent loss of Me radical yields the ion (**f**) at *m/e* 525. An alternative decomposition of (**e**) leads to the fragment (**g**, *m/e* 331) representing the sugar residue. This oxonium ion, characteristic of the glycosides of the tetraacetyl hexoses,[9, 10] gives by stepwise elimination of the acetate groups the well-known[9, 11] fragment ions at *m/e* 271, 169 and 109. The elemental composition of all the fragments discussed were substantiated by high resolution mass measurements.

These observations lead to the structure and stereochemistry of neoandrographolide as **2**.

In an attempted correlation of neoandrographolide (**2**) and andrographolide (**1**), deoxyandrographolide (**16**)[2] was converted to the tritylether (**17**) and thence to the keto tritylether (**18**) by Jones oxidation. Treatment of **18** with ethane dithiol and BF_3-etherate gave the thioketal (**19**) together with the sulphur compounds (**20** and **21**). Reduction of the thioketal (**19**) with deactivated Raney nickel led to a complex mixture which could not be separated.


Relative Abundance
100
50
m/e
43
69
109
303
331
349
271
289
525
540
635
650
100
200
300
400
480
500
600



SCHEME 2

A successful interrelationship was achieved through the ketoacetate (**22**) obtained on treatment of the keto trityl ether (**18**) with acetic anhydride and sulphuric acid. A Clemmensen reduction using the conditions described by Toda, *et al.*[12] led to no useful result. However, reduction *via* the *p*-tosylhydrazone (**23**)[13] gave, in poor yield, a compound identical with the iso-aglucone (**12**) obtained from neoandrographolide (**2**).

EXPERIMENTAL

M.ps were determined on a Kofler hot stage apparatus and are uncorrected. UV data are for EtOH solns. IR spectra for Nujol mulls and rotations in $CHCl_3$ solns unless stated otherwise. NMR spectra were done in $CDCl_3$ with TMS as internal reference. The mass spectra were obtained with the AEI MS9 and MAT CH4 using direct insertion (ion source 200° and 70° respectively) at 70 eV. High resolution mass measurements were made with the MS 9 at a resolving power of 12,000.

Isolation of neoandrographolide. After separation of andrographolide from the $CHCl_3$ extract of *Andrographis paniculata* Nees most of the solvent was removed and the resulting syrup was stirred repeatedly with benzene. The residue obtained from decantation of the benzene extracts was dissolved in EtOH and decolourized with charcoal. Concentration of the ethanolic soln and cooling afforded crude neoandrographolide, 2·20 g. from the extraction of 4·5 kg of crushed plant material.

Neoandrographolide recrystallized from EtOH as needles, m.p. 167–168° (reported[3] 174–174·5°). $[\alpha]_D$ − 48° (*c*, 1·0 in pyridine). λ_{max} 205 nm (ε 10,400) ν_{max} 3290, 1748, 1639, 900 cm^{-1} (Found: C, 65·3; H, 8·6; O, 26·1. $C_{26}H_{40}O_8$ requires: C, 65·0; H, 8·4; O, 26·6%).

After heating under reflux with ethanolic–NNaOH for 30 min neoandrographolide was recovered quantitatively on acidification of the alkaline soln.

Neoandrographolide tetraacetate (**3**). This was obtained with Ac_2O–Py overnight at room temp. The product was isolated by dilution with water and filtration. The yield of crystalline product was quantitative. The *tetraacetate* (**3**) recrystallized from EtOH in needles, m.p. 155–157°. $[\alpha]_D$ − 31° (*c*, 0·93). λ_{max} 205 nm (ε 10,300). ν_{max} 1742, 1639, 909 cm^{-1}. δ 0·68, 0·93 (CMe), 2·00, 2·02, 2·07 (3H, 6H, and 3H respectively, 4 × OAc), 3·17, 3·93 (AB system, J = 9·5 Hz, 2 × H-19), 7·10 (1H, m, $W\frac{1}{2}$ 4 Hz, H-14) (Found: C, 62·9; H, 7·4. $C_{34}H_{48}O_{12}$ requires: C, 62·95; H, 7·5%).

Hydrolysis of the tetraacetate (**3**). A soln of the tetraacetate (52 mg) and $KHCO_3$ (100 mg) in MeOH (5 ml) and water (2 ml) was kept at room temp for 20 hr. The ppt obtained on removal of the MeOH *in vacuo* and dilution with water was collected. Recrystallization from MeOH gave neoandrographolide, 40 mg (m.p., m.m.p. and IR comparison).

Tetrahydroneoandrographolide. Neoandrographolide (201 mg) was hydrogenated in EtOH (45 ml) with 10% Pd-C (192 mg). There was an uptake of 2·18 moles H_2. Working up gave a gum which on treatment with EtOH furnished the *tetrahydro derivative* (**4**) as plates, 140 mg, m.p. 98–100°. $[\alpha]_D$ − 40° (*c*, 0·95). ν_{max} 3226, 1767 cm^{-1} (Found: C, 64·7; H, 9·2. $C_{26}H_{44}O_8$ requires: C, 64·4; H, 9·15%).

The derived *acetate*, prepared with Ac_2O–Py, crystallized from aqueous EtOH as needles, m.p. 139–142°. $[\alpha]_D$ − 37° (*c*, 0·97). ν_{max} 1761, 1748 cm^{-1} (Found: C, 62·6; H, 8·1. $C_{34}H_{52}O_{12}$ requires: C, 62·6; H, 8·0%).

The norketone acetate (**6**)

(a) A soln of OsO_4 (100 mg) and **3** (200 mg) in dry dioxan (12 ml) was stored for 4 days. The osmate ester was decomposed by dry H_2S, the mixture filtered and the product extracted into EtOAc. Chromatography on silica and elution with $CHCl_3$ gave the *diol* (**5**), 120 mg, needles from aqueous MeOH, m.p. 143–145°. $[\alpha]_D$ − 19° (*c*, 0·83). λ_{max} 207 nm (ε 8000). ν_{max} 3333, 1745 cm^{-1} (Found: C, 59·2; H, 7·2. $C_{34}H_{50}O_{14}$ requires: C, 59·8; H, 7·4%). A soln of **5** (474 mg) and $NaIO_4$ (280 mg) in EtOH (14 ml) and water (8 ml) was kept at 4° for 23 hr then diluted with water (20 ml) and extracted with EtOAc (2 × 30 ml). The aqueous layer was distilled and the distillate collected in a saturated soln of 2,4-dinitrophenylhydrazine in 2N H_2SO_4. The ppt was filtered off after 3 hr and recrystallized from EtOH to give yellow needles (51 mg, 33%), m.p. 166°, identified as formaldehyde 2,4-DNP by m.m.p. and spectral comparison.

The EtOAc extract from above was evaporated to give a gum (380 mg) which was dissolved in benzene and filtered through a short column of alumina. Evaporation of the solvent and crystallisation of the residue from aqueous MeOH afforded the *norketone acetate* (**6**) as plates, 260 mg, m.p. 145–147° (dec). λ_{max} 207 nm (ε 8950). ν_{max} 1754–1739, 1701 cm^{-1}. δ 0·7, 1·05 (CMe), 1·98, 2·00, 2·05 (3H, 6H and 3H respectively, 4 × OAc).

4·45 (1H. d. J = 7·5 Hz. H-1'). 7·15 (1H. m. $W\frac{1}{2}$ 4 Hz. H-14) [Found: C. 60·6; H. 6·75; O. 32·7%; M (mass spectrum) 650·2924. $C_{33}H_{46}O_{13}$ requires: C. 60·9; H. 7·1; O. 32·0%; M. 650·2938].

(b) OsO_4 (20 mg) was added to a stirred soln of **3** (300 mg) in dioxan (10 ml) and water (2 ml) at room temp. The OsO_4 was completely dissolved after 15 min and $NaIO_4$ (2·00 g) was added in portions over 30 min. After stirring overnight. the mixture was colourless with a ppt of $NaIO_3$. The mixture was taken up in ether (100 ml) and water (100 ml) and the ethereal soln was washed with water. saturated with H_2S and filtered. The filtrate was washed with 10% $NaHCO_3$. dried and evaporated. The residue crystallised from aqueous MeOH to give **6** identical with that described above.

The Tetrol (**7**). A soln of **6** (150 mg) in EtOH : water (3 : 1. 12 ml) containing $NaHCO_3$ (300 mg) was kept at room temp for 40 hr. The mixture was then passed through Dowex 50W–X4 resin (acid form) previously washed with 3 : 1 EtOH–water. The eluate was evaporated to a small volume *in vacuo*. dilute HCl (5 drops) was added and the soln warmed on a steam bath to complete lactonisation. After cooling. the product was collected by filtration and recrystallised from EtOAc-light petroleum to give needles. 130 mg. m.p. 137–139°. λ_{max} 206 nm (ε 7700). ν_{max} 3333. 1761. 1724 cm^{-1} (Found: C. 61·7; H. 7·8. $C_{25}H_{38}O_9$ requires: C. 62·2; H. 7·9%).

Acetylation of **7** with Ac_2O–Py in the usual way afforded **6** in good yield.

Oxidation of neoandrographolide tetraacetate with potassium permanganate and sodium periodate

A soln of **3** (1·045 g). K_2CO_3 (1·20 g). $KMnO_4$ (1·62 g) and $NaIO_4$ (6·38 g) in water (120 ml) and dioxan (200 ml) was allowed to stand at room temp. After 6½ hr a further quantity of $KMnO_4$ (360 mg) was added. After 26 hr. the mixture was acidified with dil H_2SO_4 and extracted with EtOAc (2 × 200 ml). The combined extract was washed successively with water (2 × 100 ml) and 10% $NaHCO_3$ (2 × 100 ml). Acidification of the bicarbonate extract with dil H_2SO_4 and extraction into EtOAc gave. after removal of the solvent *in vacuo*. an acid fraction (380 mg) as a foam. This was methylated in the usual way with diazomethane to furnish the *methyl ester* (**9**). needles from aqueous MeOH. m.p. 142–144°. ν_{max} 1745. 1710 cm^{-1}. δ 0·77. 1·01 (CMe). 2·01 and 2·15 (9H and 3H respectively. 4 × OAc). 3·66 (3H. CO_2Me) (Found: C. 59·9; H. 7·0. $C_{31}H_{46}O_{13}$ requires: C. 59·4; H. 7·4%).

Sodium periodate oxidation of neoandrographolide and the tetrahydro derivative

(a) A mixture of neoandrographolide (100 mg) in EtOH (20 ml) and 0·01N $NaIO_4$ (50 ml) was kept at 0° in the dark. The progress of the reaction was followed by titration in the usual way. There was an uptake of 1·58 moles of periodate after 48 hr.

(b) A similar estimation using **4** showed an uptake of 1·70 moles after 48 hr.

Reaction of neoandrographolide with acetaldehyde

A mixture of neoandrographolide (100 mg). freshly fused $ZnCl_2$ (93 mg) and acetaldehyde (5 ml) was stored overnight at room temp. Most of the acetaldehyde was removed by evaporation. the residue was diluted with water and the product collected by filtration. Crystallization from aqueous EtOH and then from EtOAc–hexane gave the *acetylidene* (**10**) as needles. m.p. 199–201°. ν_{max} 3400. 3220. 1754. 1639. 893 cm^{-1} (Found: C. 67·1; H. 8·4. $C_{28}H_{42}O_8$ requires: C. 66·4; H. 8·4%).

The *acetylidene diacetate* (**11**). prepared with Ac_2O–Py. crystallized as prisms from EtOAc–hexane. m.p. 166–167°. ν_{max} 1754. 1644. 890 cm^{-1}. δ 0·66. 0·92 (CMe). 1·32 (3H. d. J = 6 Hz. *sec*-Me). 2·03. 2·08 (2 OAc). 7·12 (1H. m. $W\frac{1}{2}$ = 4 Hz. H-14). (Found: C. 65·4; H. 7·8. $C_{32}H_{46}O_{10}$ requires: C. 65·1; H. 7·85%).

Reaction of neoandrographolide with hydrochloric acid

The iso-aglucone (**12**). A soln of neoandrographolide (1·00 g) in EtOH (200 ml). water (50 ml) and conc HCl (40 ml) was heated under reflux for 5 hr. After cooling and dilution with water. the product was extracted into EtOAc (3 × 100 ml). The combined extract was washed thoroughly with water. dried and evaporated to give a gum which crystallized from aqueous MeOH as prisms. 500 mg. m.p. 121–123°. ν_{max} 3448. 1740 cm^{-1} [Found: C. 75·3; H. 9·5; O. 15·2%; M (mass spectrometry). 318·2195. $C_{20}H_{30}O_3$ requires: C. 75·4; H. 9·5; O. 15·1%; M. 318·2195].

The iso-aglucone acetate (**13**)

(a) Conc H_2SO_4 (2 drops) was added to a soln of neoandrographolide (91 mg) in HOAc (2 ml). After 15 min at room temp. the soln which was now a pale purple was heated on a water bath for 15 min. The product was recovered by dilution with water and extraction into EtOAc. The combined extract was

washed successively with water, 10% $NaHCO_3$ and water. Evaporation *in vacuo* gave a gum which was dissolved in benzene and chromatographed on silica (4 g). Elution with benzene gave the *title compound* which crystallized as plates from aqueous EtOH. 47 mg. m.p. 111–113°. $[\alpha]_D$ − 61° (*c*. 1·11). ν_{max} 1754 and 1730 cm^{-1}. δ 0·97 (6H. 2 × CMe). 1·62 (vinylic Me). 2·05 (OAc). 4·10 (2H. q. J_{AB} = 11·5 Hz. ν_{AB} = 19·6 Hz. C$\underline{H}_2$OAc). 4·78 (2H. d. J = 1·3 Hz. H-15). 7·17 (1H. $W\frac{1}{2}$ 4 Hz. H-14) [Found: C. 72·8; H. 8·95; O. 17·8%; *M* (mass spectrum) 360·2303. $C_{22}H_{32}O_4$ requires: C. 73·3; H. 8·95; O. 17·75%; *M*. 360·2300].

(b) Acetylation of **12** with Ac_2O-Py overnight at room temp gave **13** identical by m.m.p. and spectral data.

Treatment of andrographolide with acetic acid and sulphuric acid

The triacetate (**14**). Andrographolide (314 mg) was treated with HOAc and conc H_2SO_4 under conditions described in experiment (a). The product showed one major spot on TLC and was purified by PLC (3 : 1 $CHCl_3$: EtOAc as developing solvent) to give the *triacetate* (**14**). 154 mg. which crystallized as plates from MeOH. m.p. 139–141°. ν_{max} 1760. 1720 and 1660 cm^{-1}. δ 1·02. 1·05 (CMe). 1·59 (vinylic Me). 2·07. 2·17 (6H and 3H respectively. 3 × OAc). 3·03 (2H. bd. J = 7 Hz. 2 × H-11). 6·92 (1H. bt. J = 7 Hz. H-12) (Found: C. 65·1; H. 7·55. $C_{26}H_{36}O_8$ requires: C. 65·5; H. 7·6%).

The Trityl Ether (**17**). A soln of **16**[2] (6·4 g) and trityl chloride (8·0 g) in dry pyridine was heated under reflux for 42 hr then poured into water. The product, recovered with EtOAc, was triturated with 1 : 1 benzene–light petroleum (6 × 50 ml) leaving a residue which chromatographed on alumina. Elution with benzene–light petroleum (1 : 1) gave the *trityl ether* (**17**) which crystallized in plates from $CHCl_3$–light petroleum. 6·64 g. m.p. 230–232°. ν_{max} 3300. 1750 cm^{-1} (Found: C. 81·0; H. 7·75. $C_{39}H_{44}O_4$ requires: C. 81·2; H. 7·7%).

The keto trityl ether (**18**). Jones reagent was added dropwise with stirring to a soln of the above trityl ether (2·0 g) in acetone (750 ml) at 0° until a brown colour persisted. The product, recovered with EtOAc, crystallized from MeOH in prisms. 1·74 g. m.p. 194–196°. ν_{max} 1750. 1700 cm^{-1}. δ 0·43. 1·38 (CMe). 3·10. 3·45 (AB system. J = 9·5 Hz. 2 × H-19). 4·65 (H-17 overlapping with 2 × H-15 at 4·68). 4·85 (other H-17). 7·01 (1H. m. $W\frac{1}{2}$ 5 Hz. H-14). 7·32 (15H. m. $W\frac{1}{2}$ 8 Hz. aromatic protons) (Found: C. 81·8; H. 7·4. $C_{39}H_{42}O_4$ requires: C. 81·5; H. 7·4%).

The thioketal (**19**). A soln of **18** (430 mg). BF_3/Et_2O (1 ml) and ethanedithiol (1 ml) in gl HOAc (18 ml) was kept at room temp for 12 hr. The *disulphide* (**20**) crystallised from the mixture as needles (48 mg). m.p. 182–184° (from $CHCl_3$/MeOH). ν_{max} 1585 cm^{-1}. δ 7·25 (30H. m. ArH). 2·12 (4H. s. C$\underline{H}_2$C$\underline{H}_2$) (Found: C. 82·95; H. 6·0; S. 11·1. $C_{40}H_{34}S_2$ requires: C. 83·0; H. 5·9; S. 11·1%).

Dilution of the filtrate, after removal of the disulphide, with water until no more crystals were formed gave a ppt (167 mg) which on PLC in light petroleum (2 developments) yielded the disulphide (45 mg) and the *thiol* (**21**). (107 mg). needles from $CHCl_3$/MeOH. m.p. 116–120°. ν_{max} 2510. 1570 cm^{-1}. δ 7·32 (15H. m. Ar$\underline{H}$). 2·0–2·6 (4H. complex m. C$\underline{H}_2$C$\underline{H}_2$). 1·38 (1H. t. J = 7·5 Hz. S$\underline{H}$) (Found: C. 74·1; H. 5·9; S. 19·2. $C_{21}H_{20}S_2 \cdot \frac{1}{4}H_2O$ requires: C. 73·9; H. 5·9; S. 18·8%).

The filtrate was further diluted with water and extracted with EtOAc to yield a gum (333 mg) which on PLC in $CHCl_3$ gave the *thioketal* (**19**) (150 mg). needles from MeOH/water. m.p. 158–162°. ν_{max} ($CHCl_3$) 3500. 1751. 900 cm^{-1}. δ 0·67. 1·22 (CMe). 3·27 (4H. bs. $W\frac{1}{2}$ 3 Hz. C$\underline{H}_2$C$\underline{H}_2$). 3·54. 3·88 (AB system. J = 12 Hz. 2 × H-19). 4·61. 4·87 (1H each. s. C=C$\underline{H}_2$). 4·78 (2H. m. $W\frac{1}{2}$ 5 Hz. 2X H-15). 7·14 (1H. m. $W\frac{1}{2}$ 5 Hz. H-14) (Found: C. 64·4; H. 7·7; S. 15·8. $C_{22}H_{32}O_3S_2$ requires: C. 64·7; H. 7·9; S. 15·7%).

The keto acetate (**22**). A mixture of **18** (900 mg). HOAc (25 ml) and conc H_2SO_4 (0·7 ml) was heated on a water bath for 15 min then poured into cold 10% $NaHCO_3$ aq. The ppt was collected and crystallized from MeOH aq to give the *ketoacetate* (**22**) as prisms. 263 mg. m.p. 101·5–104°. ν_{max} 1750. 1700. 1250 cm^{-1}. δ 1·20 (2 × CMe). 1·66 (vinylic Me). 2·00 (OAc). 3·98. 4·60 (AB system. J = 11·5 Hz. 2 × H-19). 4·80 (2H. m. $W\frac{1}{2}$ = 5 Hz, 2 × H-15). 7·16 (1H, m. $W\frac{1}{2}$ = 5 Hz, H-14). (Found: C, 70·2; H, 8·2, $C_{22}H_{30}O_5$ requires: C, 70·6; H, 8·1%).

The tosylhydrazone (**23**). A soln of **22** (200 mg) and *p*-toluenesulphonylhydrazine (115 mg) in AcOH (10 ml) was set aside at room temp overnight. The ppt obtained on pouring the mixture into water was collected and crystallized from MeOH in prisms. 208 mg. m.p. 153–157°. ν_{max} 1760. 1735 cm^{-1}. δ 1·04. 1·16 (CMe). 1·62 (vinylic Me). 1·70 (OAc). 2·42 (ArMe). 3·75. 4·50 (AB system. J = 11 Hz. 2 × H-19). 4·79 (2H. m. $W\frac{1}{2}$ = 4 Hz. 2 × H-15). 7·20 (1H. m. H-14). 7·33. 7·88 (2H each. d. J = 8 Hz. ArH). *ca* 7·85 (exchangeable with D_2O. NH) (Found: C. 64·0; H. 6·9. $C_{29}H_{38}O_6N_2S$ requires: C. 64·2; H. 7·0%).

Reduction of the tosylhydrazone (**23**). A mixture of the tosylhydrazone (80 mg). NaOH (400 mg) and $NaBH_4$ (1·50 g) in 65% aqueous diglyme was stirred at room temp for 17 hr. The mixture was poured into

cold dil HCl and the product recovered with $CHCl_3$. Purification of the product by PLC (using a sample of **12** as marker) and crystallisation from aqueous EtOH gave plates, 15 mg, m.p. 120–122°, identical with **12** by m.m.p., TLC and IR spectral comparison.

Acknowledgements—We thank Dr. G. Snatzke for obtaining the CD data and for helpful comments. Alcan Jamaica Ltd., for a Junior Research Fellowship (to C.R.W.) and the Research and Publications Fund. University of the West Indies, for providing the V-6058 spin decoupler. One of us (H.-W.F) also thanks the Stiftung Volkswagenwerk for supplying the mass spectrometers.

REFERENCES

[1] G. F. Asprey and P. Thornton. *W.I. med. J.* **2**. 233 (1953); R. N. Chopra, I. C. Chopra, K. L. Handa and L. D. Kapur. *Indigenous Drugs of India* p. 278. U. N. Dhur. Calcutta (1958)
[2] M. P. Cava, W. R. Chan, R. P. Stein and C. R. Willis. *Tetrahedron* **21**. 2617 (1965), and refs cited
[3] R. J. C. Kleipool. *Nature. Lond.* **69**. 33 (1952)
[4] For a preliminary communication of some of these results see W. R. Chan, D. R. Taylor, C. R. Willis and H.-W. Fehlhaber. *Tetrahedron Letters* 4803 (1968)
[5] N. S. Bhacca, L. F. Johnson and J. N. Shoolery. *NMR Spectra Catalog* Vol. I. No. 51. Varian Associates. Palo Alto. California (1962)
[6] R. Pappo, D. S. Allen, Jr., R. U. Lemieux and W. S. Johnson. *J. Org. Chem.* **21**. 478 (1956)
[7] R. U. Lemieux and E. von Rudloff. *Canad. J. Chem.* **33**. 1701 (1955)
[8] H. Budzikiewicz, D. H. Williams and C. Djerassi. *Structure Elucidation of Natural Products by Mass Spectrometry* Vol. II. p. 117. Holden-Day. San Francisco (1964)
[9] K. Heyns, H. F. Grützmacher, H. Scharmann and D. Müller. *Fortschr. chem. Forsch.* **5**. 448 (1966)
[10] I. A. Pearl and S. F. Darling. *Phytochem.* **7**. 831 (1968)
[11] K. Heyns and D. Müller. *Tetrahedron Letters* 6061 (1966)
[12] M. Toda, Y. Hirata and S. Yamamura. *Chem. Comm.* 919. (1969)
[13] L. Cagliotti. *Tetrahedron* **22**. 487 (1966)