THE STRUCTURE OF ANDROGRAPHOLIDE

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Abstract-Evidence is presented for the structure [II] for andrographolide.

ANDROGRAPHOLIDE, $C_{20}H_{30}O_5$, the main crystalline bitter principle of Andrographis paniculata Nees (known in the West Indies as "rice bitters") was first isolated by Gorter,¹ and has been the subject of a number of chemical investigations.²⁻⁵

Some of the previous relevant work has shown that andrographolide contains three hydroxyl groups, a methylene group and an α,β -unsaturated lactone function. On selenium dehydrogenation, andrographolide affords 1,2,5,6-tetramethylnaphthalene which suggested that it was a bicyclic diterpenoid lactone with a hydroxyl group at C_a (as in I).

New evidence is now presented which, together with previously known data, defines the structure of andrographolide as II.⁶

Acetylation of andrographolide with acetic anhydride and zinc chloride affords, in good yield, triacetylandrographolide (III). This is a true derivative of andrographolide since it is reconverted to the parent compound by mild treatment with alkali. Hydroxylation of triacetylandrographolide with osmium tetroxide in dioxan, gave a diol (IV) or a tetrol (V) according to the conditions employed. That the formation of the diol involved the isolated methylene group was evident from the ultra-violet spectrum, which showed unaltered high intensity absorption at low wave lengths, and the disappearance from the infra-red spectrum of the bands associated with the methylene group. Periodate cleavage of the diol gave, in good yield, formaldehyde and a nor-ketone, $C_{25}H_{34}O_9$, formulated as VI.

The nature of the carbocyclic ring system in andrographolide was demonstrated as follows. According to Schwyzer *et al.*,² oxidation of triacetylandrographolide either with ozone or potassium permanganate in acetone gave a methyl keto ester, m.p. $119.5-120^{\circ}$, which they considered to be $C_{19}H_{28}O_7$. We have confirmed the formation of this ester and established the identity by mixed melting point determination with a sample kindly supplied by Dr. Schwyzer. However, analytical results for this ester

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¹ M. K. Gorter, Rec. Trav. Chim. 30, 151 (1911).

- ⁸ R. J. C. Kleipool and D. G. F. R. Kostermans, *Rec. Trav. Chim.* 70, 1085 (1951); R. J. C. Kleipool, *Nature, Lond.* 169, 33 (1952).
- ⁴ D. Chakravarti and R. N. Chakravarti, J. Chem. Soc. 1697 (1952).
- ⁶ H. Kondo and A. Ono, Ann. Rep. Itsuu Lab. 9, 85 (1958) and earlier papers.
- ⁶ For preliminary communications of these results, see: M. P. Cava and B. Weinstein, *Chem. & Ind.* 851 (1959); W. R. Chan, L. J. Haynes and L. F. Johnson, *Ibid.* 22 (1960).

² R. Schwyzer, H. G. Biswas and P. Karrer, *Helv. Chim. Acta* 34, 652 (1952) and references cited therein.

and the derived 2,4-dinitrophenylhydrazone gave values in good agreement for the formula $C_{20}H_{30}O_7$. The ester may be prepared in better yield, and under essentially neutral conditions, by diazomethane methylation of the acid (VII) obtained by ozonolysis of triacetylandrographolide followed by oxidation of the ozonide with hydrogen peroxide.

The structure VIII is assigned to the ester for the following reasons. It has two acetyl groups, gives no ferric chloride colour and on selenium dehydrogenation affords



1,2,5,6-tetramethylnaphthalene and 1,5-dimethyl-2-naphthol² indicating that it contained the bicyclic nucleus, while the optical rotatory dispersion curve⁷ is consistent with that of a *trans*-2-decalone. The relationship of the ketone carbonyl and the carboxyl group was shown in the following way. Reduction of the acid (VII) with sodium borohydride gave the corresponding hydroxy acid, which was not isolated but was transformed on heating or by a trace of mineral acid to a product which was shown by its infrared spectrum to be a γ -lactone, as in IX. The further substitution on the bicarbocyclic nucleus was evident from the following experiments. Tritylation of andrographolide by refluxing with trityl chloride and pyridine gave an anhydrotrityl ether (X) in which the allylic hydroxyl group was eliminated. This compound

⁷ We would like to express our best thanks to Professor C. Djerassi for the dispersion curves obtained during this work.

on oxidation with chromium trioxide in pyridine afforded the ketone XI (ν_{max} 1770: unsaturated y-lactone; 1715: cyclohexanone C=O) which on treatment with sulfuric acid generated formaldehyde by a retroaldol reaction for which there are many analogies.⁸ In conformity with this evidence for the structure (VIII) for the keto ester, the nuclear magnetic resonance spectrum of triacetylandrographolide (in CDCl₃) obtained at 60 megacycles and referenced to internal tetramethylsilane shows two tertiary methyl signals at $\delta = 0.75$ and $\delta = 1.02$.⁹ In addition there are three acetate signals, two superimposed at $\delta = 2.03$ and one at $\delta = 2.10$.

Definite evidence of the substitution and immediate environment of the α,β -unsaturated lactone function in triacetylandrographolide was also obtained from the above spectrum. Thus a signal at $\delta = 5.91$ is characteristic of a proton attached to a carbon atom to which is attached an acetate group and another doubly bonded carbon atom, while a triplet signal centred around $\delta = 6.97$ is typical of a proton on a doubly bonded carbon atom which is β to, and conjugated with, a carbonyl group. The fact that this signal is tripled with a 6 c.p.s. spin-spin coupling constant indicates that there are two protons on the adjacent carbon atom. This interpretation leads to the partial structure (XII) for the lactone moiety.

The infra-red spectrum of andrographolide in Nujol and in KBr shows a carbonyl band at 1727 cm⁻¹ and this was taken by Kleipool³ to indicate the presence of an α,β unsaturated γ -lactone. We now find that in acetonitrile solution, andrographolide shows a band at 1754 cm⁻¹ which is in better agreement with such a system.¹⁰ Moreover, tetrahydroandrographolide, obtained by catalytic reduction of andrographolide in a neutral medium,² shows normal saturated γ -lactone absorption at 1775 cm⁻¹. Taken in conjunction with the nuclear magnetic resonance data quoted above, this leads to the formulation of the lactone system as in XIII, and with the evidence quoted above leads to the formulation of andrographolide as II.

Reduction of triacetylandrographolide with aluminium amalgam affords desoxyandrographolide diacetate. We regard this compound as having structure XIV, arising as shown in XV, although the alternate formulation XVI is not rigidly excluded. Oxidation with potassium permanganate in acetone followed by methylation of the acidic product gave the keto-ester (VIII). The desoxy diacetate also shows strong end absorption in the ultra-violet which is analogous with that shown by other α,β -unsaturated lactones; and in the infra-red spectrum, a band at 1756 cm⁻¹ is in satisfactory agreement with an α,β -unsaturated δ -lactone system. Moreover, it shows a characteristic colour reaction (Legal test) of α,β -unsaturated δ -lactones. In agreement with XIV, the low field nuclear magnetic resonance of the compound in deuterochloroform, shows only a group of signals centred around $\delta = 7.13$ which are characteristic of a β -proton in an α,β -unsaturated ketone or ester. These signals are not characteristic of an α -proton, and as a model compound, the spectrum of crotonic acid shows the α -hydrogen signals centred around $\delta = 5.88$ while the β -hydrogen signals are centred around $\delta = 7.17$.

The absolute configuration of the ring junction and the attachment of the side

 ^a cf. D. H. R. Barton and P. deMayo, J. Chem. Soc. 887 (1954); C. Djerassi and W. Rittel, J. Amer. Chem. Soc. 79, 3528 (1957); D. H. R. Barton, S. K. Pradhan, S. Sternhell and J. F. Templeton, J. Chem. Soc. 255 (1961).

[•] See Djerassi, et al. J. Org. Chem. 26, 1192 (1961) for definition of δ and reasons for its use rather than τ . The relationship $\tau = 10 - \delta$ serves to convert from one system to the other.

¹⁰ We thank Dr. K. H. Overton of the University of Glasgow for this spectrum.

chain follows from a consideration of the optical rotatory dispersion measurements on the nor-ketone (VI) and the keto ester acetate (VIII). They are both characterized by a strong, positive, single Cotton effect which is not only a mirror image of that shown by friedelin¹¹ and the keto ester (XVII) derived from labdanolic acid,¹² but also



a duplicate of that of a 2-keto- 5α -steroid.¹³ These rotatory dispersion curves are in agreement with the absolute representation (VIII) for the keto ester which indicates that andrographolide belongs to the class of terpenoids, of which iresin¹⁴ was the first example, having a configuration which is the mirror image of that found in the steroids and most of the higher terpenoids. The presence of a hydroxyl group at C₃ is

- ¹³ J. D. Cocker and T. G. Halsall, J. Chem. Soc. 4262 (1956).
 ¹³ C. Djerassi, W. Clossen and A. E. Lippman, J. Amer. Chem. Soc. 78, 3136 (1956).
- 14 C. Djerassi and S. Burstein, Tetrahedron 7, 37 (1959).

¹¹ C. Djerassi, R. Riniker and B. Riniker, J. Amer. Chem. Soc. 78, 6362 (1956).

significant and biogenetic speculations¹⁵ have already been advanced. The high intensity of the Cotton curves is used to assign the equatorial configuration to the side chain of the ester, since it has been established^{11,16} that in an antipode pair, axial substitution adjacent to a ring junction leads to a marked reduction in the amplitude of the rotatory dispersion curve without inverting the sign of the single Cotton effect curve.

Andrographolide is recovered unchanged after refluxing in acetone with *p*-toluene sulfonic acid for four days. Similar attempts at the formation of acetylidene and benzylidene derivatives under conditions where *cis*-1,3-diols readily gave such derivatives were also without success. This suggests a *trans*-relationship between the C_3 -hydroxyl and the primary alcohol at C_4 .

Further work on the chemistry of andrographolide is in progress.

EXPERIMENTAL

M.p. were determined on a Kofler block and are uncorrected. Ultra-violet spectra were recorded on a Beckmann spectrophotometer and are for ethanol solutions unless otherwise stated. Infra-red spectra were obtained an a Perkin-Elmer Model 21 spectrophotometer and are for KBr discs unless otherwise stated. The alumina used for chromatography was neutral and graded according to the Brockmann scale of activity.

Andrographolide

This was recrystallized from either ethanol or methanol as plates, m.p. 230–231°, $[\alpha]_D - 127°$ (c, 1.02 in acetic acid). Reported:^a m.p. 227.5° $[\alpha]_D - 126.6°$. λ_{max} 223 m μ [ϵ 12,300], ν_{max} 3448 and 3390–3279 (OH), 1828, 1647 and 906 (exocyclic methylene), 1727 (α,β -unsaturated γ -lactone), 1672 (conjugated C=C) cm⁻¹. (Found: CMe, 7.50. 2CMe in C₂₀H₃₀O₅ requires: 8.58%).

Triacet ylandrographolide

This was prepared by the action of acetic anhydride and zinc chloride on andrographolide as described previously.⁴ It had $[\alpha]_D -94^\circ$ (c, 1.03 in CHCl₃), $\lambda_{max} 224 \text{ m}\mu$ ($\epsilon 12,900$), $\nu_{max} 1764-1742$ (acetate C=O and α,β -unsaturated γ -lactone), 1692 (conjugated C=C), 900 (exocyclic methylene) cm⁻¹. (Found: CMe, 15.34. 5CMe in C₂₆H₃₆O₈ requires: 15.75%).

Hydrolysis of triacetylandrographolide

The triacetate (75 mg) was stirred overnight with a mixture of 2N sodium hydroxide (2 ml) and ethanol (3 ml). The solution was evaporated *in vacuo*. The residue was dissolved in water (3 ml), acidified with excess hydrochloric acid and heated on a water bath. Recrystallization of the gummy precipitate from methanol gave andrographolide (39 mg) identified by infra-red spectral comparison.

Diacetyldeoxyandrographolide

This was prepared as previously described, ⁴ m.p. 120° (reported 118°, 120°), $[\alpha]_D - 26^\circ$ (c, 1.22 in CHCl₃), λ_{max} 214 m μ (ϵ 9,300), 220 m μ (ϵ 8,500), 230 m μ (ϵ 4,400); ν_{max} 1764 [lactone], 1727 [ester carbonyls], 1647 and 907 (exocyclic methylene) cm⁻¹. (Found: CMe, 12.85, 4CMe in C₃₄H₃₄O₈ requires: 14.36%).

Hydrolysis gave deoxyandrographolide, m.p. 168° (reported* 170°), $[\alpha]_D - 31°$ (c, 1·22 in CHCl_a), ν_{max} 3344–3279 [OH], 1764 [γ -lactone], 1639 and 899 [exocyclic methylene] cm⁻¹.

Hydroxylation of triacetylandrographolide

(a) The triacetate (721 mg) and osmium tetroxide (424 mg) in dry dioxan (15 ml) was allowed to stand at room temp. for 2 days. The osmate ester was decomposed with hydrogen sulphide. Filtration followed by evaporation of the filtrate *in vacuo* gave a colourless gum which crystallized from ethyl acetate-hexane to give the *diol* (IV), 290 mg, m.p. 173-174°, λ_{max} 221 m μ (ϵ 10,720), ν_{max} 3534-3460 (OH), 1770-1724 (acetate and α,β -unsaturated lactone C=O), 1680 (conjugated C=C) cm⁻¹. (Found: C, 60.94; H, 7.60. C₂₈H₃₈O₁₀ requires: C, 61.16; H, 7.50%).

¹⁵ C. Djerassi, M. Cais and L. A. Mitscher, J. Amer. Chem. Soc. 81, 2386 (1959).

¹⁶ C. Djerassi, O. Halpern, V. Halpern and B. Riniker, J. Amer. Chem. Soc. 80, 4001 (1958).

(b) A solution of the triacetate (502 mg) and osmium tetroxide (581 mg) in dry dioxan (20 ml) was allowed to stand at room temp. for 5 days. Crystallization of the product from benzene and then from ethyl acetate-benzene afforded the *tetrol* (V) as prisms, m.p. 149-150°, $[\alpha]_D - 44^\circ$ (c, 1·16 in CHCl₃), no high intensity ultra-violet absorption, ν_{max} 3390 (OH), 1770-1727 (acetate and γ -lactone C=O) cm⁻¹. Found: C, 57·66; H, 7·48. Ca₂H₄₀O₁₃ requires: C, 57·34; H, 7·40%). Titting with pariadic acid chourd on untoke of 1.4 moles of the pariadic state.

Titration with periodic acid showed an uptake of 1.4 moles after 23 hr.

Ketotriacetylnorandrographolide (VI)

A solution of diol (IV, 150 mg) in methanol (5 ml) was diluted with a solution of periodic acid (160 mg) in water (3 ml). After standing for 1 hr at room temp, the mixture was cooled overnight in a refrigerator. The precipitate which formed was filtered and crystallized from accone-hexane to yield 118 mg (84%) of colourless plates, m.p. 126-127°. $\lambda_{max} 219.5 \text{ m}\mu (\epsilon 9,100) \nu_{max} 1770$ (y-lactone), 1742-1712 (acetate C=O, ketonic C=O). R.D. in methanol (c, 0.06): $[\alpha]_{700} - 30^{\circ}$, $[\alpha]_{889} - 45^{\circ}$, $[\alpha]_{422-440} - 90^{\circ}$, $[\alpha]_{410} - 90^{\circ}$ [shoulder], $[\alpha]_{323} - 170^{\circ}$, $[\alpha]_{335} - 120^{\circ}$, $[\alpha]_{540} - 40^{\circ}$ (inflection), $[\alpha]_{230} - 1810^{\circ}$. The ketone gave a deep red-brown colour in the Zimmerman test. (Found: C, 62.54; H, 7.13. $C_{25}H_{34}O_9$ requires: C, 62.75; H, 7.16%).

The original filtrate from the periodate oxidation was diluted with water (100 ml), neutralized with sodium carbonate and distilled. The distillate (50 ml) reacted with a solution of 2,4-dinitrophenylhydrazine in aqueous sulphuric acid to give a crystalline precipitate of formaldehyde 2,4-dinitrophenylhydrazone (55 mg, 83%), identified by mixed m.p. and infra-red spectrum.

Ketoester (VIII)

Triacetylandrographolide was oxidized with permanganate exactly as described previously.^{*} The crude ketoester was purified by chromatography in benzene over alumina (Grade III), followed by several crystallizations from hexane to give flat needles, m.p. 121.0-121.5°, λ_{max} 282 m μ (ϵ 33), ν_{max} 1736 (acetate C==O), 1715 (cyclohexanone C==O) cm⁻¹. R.D. in methanol (c, 0.1): $[\alpha]_{700}$ +24°, $[\alpha]_{389}$ +52°, $[\alpha]_{310}$ +1626° (inflection), $[\alpha]_{306}$ +1718° (peak), $[\alpha]_{267.5}$ -1512°. The compound gave a deep purple colour in the Zimmerman test. (Found: C, 63.02; H, 7.92; OAc, 22.2. $C_{20}H_{30}O_7$ requires: C, 62.81; H, 7.91; 2OAc, 22.5%).

The 2,4-dinitrophenylhydrazone, prepared in the usual way, crystallized from aqueous ethanol as yellow needles, m.p. 180^o. λ_{max} [CHCl₃] 364 m μ [ϵ 24,000], ν_{max} 1745 [overlapping ester C=O]. (Found: C, 55 71; H, 5 74; N, 10 10. C₂₈H₃₄N₄O₁₀ requires: C, 55 51; H, 6 09; N, 9 96%).

Keto-acid (VII)

Ozonized oxygen was bubbled through a solution of triacetylandrographolide (1:20 g) in dry ethyl acetate at -27° until a small sample gave no colour with 5% methanolic potassium hydroxide (1 hr). Excess ozone was removed *in vacuo*, 30% hydrogen peroxide (4 ml) and water (6 ml) added and the mixture shaken continuously for 16 hr. The acidic product, crystallized from aqueous ethanol, gave the keto-acid (VII), m.p. $187-189^{\circ}$, $[\alpha]_{\rm D} + 46^{\circ}$ (c, 1:24 in CHCl₃), $\nu_{\rm max}$ 1720, 1706 and 1690 (acetates, carbonyl and acid group) cm⁻¹. (Found: C, 62:10; H, 7:86; O, 29:95. C₁₉H₂₈O₇ requires: C, 61:94; H, 7:66%).

The *methylester*, prepared in the usual way with diazomethane, recrystallized from aqueous methanol (needles), m.p. 121°, was identical with the ester obtained from permanganate oxidation by mixed m.p. and infra-red spectrum.

Acetoxy lactone (IX)

A solution of the ketoacid (VII; 200 mg) and sodium borohydride (520 mg) in methanol (6.5 ml) was kept at 0° for 5 min and then at room temp for 10 min. The residue obtained on removal of solvent *in vacuo* was dissolved in water (2 ml), cooled in ice and carefully acidified with N-hydrochloric acid (Congo red) to afford the crude hydroxy acid (178 mg). Recrystallization from water gave a *product* which consisted of a mixture of the hydroxy acid and the γ -lactone (from the infra-red spectrum). It was therefore completely converted to the lactone in the following way.

The crude product (46 mg) was warmed with water (4 ml) and concentrated hydrochloric acid (2 drops). On standing the acetoxy lactone (1X) separated out slowly. Recrystallization from ethyl acetate gave needles, m.p. 209–212°, $[\alpha]_D + 28^\circ$ (c, 0.84 in CHCl₃), ν_{max} 1770 [γ -lactone,] 1727 and 1240 [acetate] cm⁻¹. (Found: C, 64.95; H, 8.02. C₁₉H₁₈O₈ requires: C, 64.75; H, 8.01%).

Alternatively, the crude hydroxy acid (7 mg) on heating to 115° in high vacuum for 20 hr afforded a residue (4 mg) identified as the lactone by m.p., mixed m.p. and infra-red spectrum.

Anhydroandrographolide trityl ether (X)

A mixture of andrographolide (1.05 g) and trityl chloride (1.85 g) in pyridine (10 ml) was heated for 2.5 hr on a steam bath and then allowed to stand overnight. The solution was poured into cold

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water (300 ml) and extracted with chloroform (3 \times 350 ml). Working up under the usual conditions afforded an amorphous residue which was dissolved in benzene and chromatographed on alumina (Grade III).

Benzene-ether (9:1) eluted 1.05 g of the crude ether which was crystallized from benzene-hexane and acetone-hexane to give small prisms m.p. 200.0-200.5°, λ_{max} 248 m μ (shoulder, ε 11,500). Subtraction of the spectrum of methyl trityl ether from the above spectrum revealed a maximum at 249 m μ (ε 11,200), v_{max} 3509 (OH), 1764 (α,β -unsaturated γ -lactone), 1709 (C=C) 1645 and 903 (exocyclic methylene), 1595 (phenyl) cm.⁻¹ (Found: C, 81.61; H, 7.74. C₃₉H₄₂O₄ requires: C, 81.50; H, 7.37%).

The trityl ether was relatively stable under the usual acidic hydrolysis conditions. A small sample was recovered almost quantitatively after heating for 2 hr with acetic acid (2 ml) containing a drop of 1% sulphuric acid.

3-Dehydroanhydrographolide trityl ether (XI)

A solution of the trityl ether (X; 510 mg) in pyridine (5 ml) was added dropwise with stirring a cold solution of chromium trioxide (350 mg) in pyridine (5 ml). After 7 hr at room temp, the mixture was diluted with methanol (10 ml). After 2 hr solvents were removed *in vacuo* at room temp and the residue triacted with acetone (10 × 25 ml). The residue remaining on evaporation of the acetone was chromatographed in benzene over alumina (Grade III). Evaporation of the benzene eluate and crystallization from acetone-hexane gave flat needles (145 mg, 29%), m.p. 205°, λ_{max} 246 m μ (shoulder; ϵ 12,900). Subtraction of the spectrum of methyl trityl ether revealed a maximum at 247 m μ , (ϵ 12,600), v_{max} 1770 (α,β -unsaturated γ -lactone), 1715 (cyclohexanone C=O), 1647 and 899 (exocyclic methylene), 1603 (phenyl) cm⁻¹. The compound gave a pale red-brown colour in the Zimmerman test. Under the same conditions 3-dehydroiresin trityl ether¹⁴ gave a pale pink colour. (Found: C, 81.52; H, 7.05. C₃₉H₄₀O₄ requires: C, 81.79; H, 7.04%). A sample of XI was heated at 100° for 6 hr with chromotropic acid-sulphuric acid reagent. A

A sample of XI was heated at 100° for 6 hr with chromotropic acid-sulphuric acid reagent. A duplicate colorimetric analysis¹⁷ showed the presence of formaldehyde (75% and 95% of one equivalent in two different experiments).

Diacetylanhydroandrographolide

A mixture of andrographolide (1.0 g), acetic anhydride (10.0 ml) and anhydrous sodium acetate (1.0 g) was placed in a preheated oil bath at 125° and stirred for 30 min. The resulting dark solution was poured into water (50 ml) and allowed to stand overnight. Crystallization of the precipitate from aqueous ethanol afforded 60 mg of yellow needles, m.p. 120-121°. Chromatography in benzene over alumina (Grade III) yielded a product which recrystallized from ethanol as white needles, m.p. 136·5-137·5° (reported 136°), $\lambda_{max} 248.5 \text{ m}\mu$ (ϵ 11,000) v_{max} 1764-1733 (y-lactone and acetate), 1695 (conjugated C=C), 1674 and 895 (exocyclic methylene), 1608 (conjugated C=C) cm⁻¹. (Found: C, 69·52; H, 7·55; CMe, 14·03. C₂₄H₂₃O₆ requires: C, 69·21; H, 7·74; 4CMe, 14·30%).

Triacetylandrographolide dibromide

This was prepared as previously described,¹ m.p. 179.5-180.0° (reported 175°), λ_{max} 220 m μ (ϵ 10,500), ν_{max} 1776 (lactone carbonyl) 1757-1733, (acetate C=O), 1681 (conjugated C=C) cm⁻¹. (Found: C, 48.88; H, 5.89; Br, 24.97. C₂₄H₃₄O₈Br₂ requires: C, 49.06; H, 5.70; Br, 25.11%).

¹⁷ A Klett colorimeter, Model 800-3, equipped with a 540 mμ filter was used for this analysis, cf. W. R. Frisell, L. A. Meeck and C. G. Mackenzie, J. Biol. Chem. 207, 709 (1954).