

**314. Andrographolide. Part I.**

By (MRS.) D. CHAKRAVARTI and R. N. CHAKRAVARTI.

Contrary to observations by Guha-Sircar and Moktader, andrographolide does not contain a methylenedioxy-group; Gorter's original findings that andrographolide is a trihydroxy-lactone, with one tertiary hydroxy-group, have been established. Hydrogenation of andrographolide in acetic acid solution with Adams's catalyst furnishes two isomeric deoxytetrahydroandrographolides, indicating the presence of two double bonds. Hydrogenation in methanolic solution in presence of palladium leads to the same mixture of isomers; Guha-Sircar and Moktader's claim to have isolated a dihydroandrographolide under these conditions was not substantiated. That andrographolide is an  $\alpha\beta$ -unsaturated lactone is evident from ultra-violet absorption spectra and a positive Legal test. Elimination of a molecule of formaldehyde during ozonolysis indicates the presence of a methylene group attached to a double bond.

*Andrographis paniculata* Nees (Bengali, Kalmegh) grows widely throughout the plains of India. Its extremely bitter leaves form the chief constituent of a household medicine, "Alui," extensively used in Bengal in cases of bowel complaints of children and as a febrifuge and anthelmintic. According to Chopra ("Indigenous Drugs of India," Art Press, Calcutta, 1933, p. 280), as a bitter it is in no way inferior to other bitters mentioned in pharmacopœias.

Pure crystalline andrographolide, the bitter constituent of this plant, was first isolated by Gorter (*Rec. Trav. chim.*, 1911, **30**, 151; 1914, **33**, 239). According to him it is a trihydroxy-lactone,  $C_{20}H_{30}O_5$ , with one tertiary hydroxy-group. Later, Guha-Sircar and Moktader (*J. Indian Chem. Soc.*, 1939, **16**, 333) failed to prepare Gorter's triacetyl-andrographolide but obtained evidence indicating the presence of a methylenedioxy-group and a hydroxy-group in andrographolide instead of three hydroxy-groups.

The present authors have confirmed Gorter's original findings mentioned above. Contrary to observations by Guha-Sircar and Moktader (*loc. cit.*) it gives no formaldehyde on distillation with 33% sulphuric acid and does not appear to contain a methylenedioxy-group: it does not respond to Gabel's test or give a green colour with gallic acid and concentrated sulphuric acid. On the other hand, as a trihydroxy-compound, under conditions described in the Experimental section, it furnishes the triacetate which Guha-Sircar and Moktader failed to prepare. We have also substantiated Gorter's observation that this triacetyl derivative on reduction with aluminium amalgam in presence of moist ether affords diacetyldeoxyandrographolide,  $C_{20}H_{28}O_2(OAc)_2$ , indicating that one of the three hydroxy-groups is tertiary.\*

Contrary to Guha-Sircar and Moktader's statements, andrographolide smoothly reduces ammoniacal silver nitrate and cold potassium permanganate. When subjected to the Legal test under the modified conditions described by Paist, Blout, Uhle, and Elderfield (*J. Org. Chem.*, 1941, **6**, 273) it appears to contain an  $\alpha\beta$ -unsaturated lactone system. This is confirmed by the ultra-violet absorption spectrum. In this respect it is similar to the other physiologically active unsaturated lactones occurring in Nature.

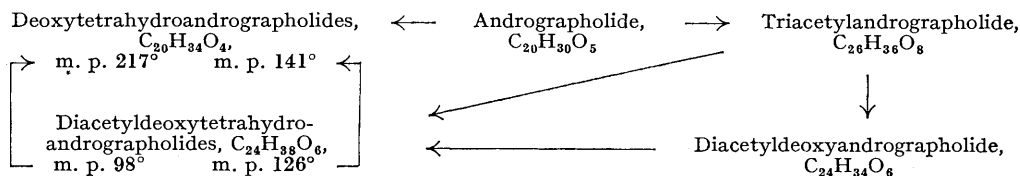
Hydrogenation of andrographolide in acetic acid solution in presence of Adams's catalyst leads to two isomeric deoxytetrahydroandrographolides,  $C_{20}H_{34}O_4$ , indicating the presence of two double bonds and a labile hydroxy-group which is eliminated. This further supports Gorter's observation (*loc. cit.*) that andrographolide contains a tertiary hydroxy-group.

\* A short communication containing all the findings described in the present paper was submitted on 15th September, 1950, and appeared in the Proceedings of the 38th Indian Science Congress, 1951, Part III, Abstracts, p. 79. Most of the findings have since been confirmed by Schwyzer, Biswas, and Karrer (*Helv. Chim. Acta*, 1951, **34**, 652). No reference was, however, made in the latter paper to our findings which have priority.

The same products are obtained by carrying out the hydrogenation in methanolic solution (acidic) in presence of palladium chloride although Guha-Sircar and Moktader (*loc. cit.*) could only isolate a dihydroandrographolide under these conditions. Thus, also with palladium hydrogenolysis of the tertiary hydroxy-group takes place.\*

The presence of two double bonds, three hydroxy-groups and the lactone system having been established, the molecule should contain two homocyclic rings.

Alkaline hydrolysis of a mixture (m. p. 175°) of the two isomers of deoxytetrahydroandrographolide, as obtained by hydrogenation of triacetylandrographolide followed by hydrolysis, affords a single crystalline product, deoxytetrahydroandrographolic acid,  $C_{20}H_{36}O_5$ , m. p. 107—108°. Gorter isolated diacetyldeoxytetrahydroandrographolide, m. p. 90—91°, by hydrogenation of diacetyldeoxyandrographolide in acetic acid solution in presence of platinum black. It has now been possible to isolate from this product two isomers, m. p. 126° and m. p. 98° respectively. Under similar conditions triacetylandrographolide absorbs three mols. of hydrogen with the formation of the same mixture of isomers of diacetyldeoxytetrahydroandrographolide, indicating loss of the acetoxy-group corresponding to the tertiary hydroxy-group. None of these deoxy-compounds gives a red colour with methanolic potassium hydroxide which is a characteristic test for andrographolide and its derivatives containing the tertiary hydroxy-group. On deacetylation the higher-melting diacetyldeoxytetrahydroandrographolide gives the lower-melting deoxytetrahydroandrographolide, the higher-melting isomer of which is obtained by the deacetylation of the product, m. p. 98°, described above (cf. scheme).



When andrographolide is ozonised in acetic acid solution and the product is distilled in steam, formaldehyde is isolated in almost quantitative yield as the 2 : 4-dinitrophenylhydrazone; a second ketone was isolated, but in quantity too small for further work.

### EXPERIMENTAL

Analyses by Dr. G. Weiler and Dr. F. B. Strauss, of Oxford.

Large quantities of andrographolide required for this work were obtained by extraction of the air-dried leaves of *Andrographis paniculata* with boiling chloroform in a Soxhlet extractor as described by Guha-Sircar and Moktader (*loc. cit.*). The crude light green product obtained in this way was purified by crystallisation from methanol, separating in large colourless prisms, m. p. 228° (frothing). Andrographolide prepared by Gorter had m. p. 218°. The seasonal variation in andrographolide contents of this annual plant is considerable. An average yield above 2% of the air-dried material can be obtained if the leaves are collected in October—November just before flowering. The yield drops to less than 0.5% after January, when the leaves also become very scarce. The stems of the plant also contain sufficient amounts of the bitter principle and the concentration in the stems runs parallel to that in the leaves. The root and seeds of this plant do not appear to contain andrographolide.

Andrographolide (Found : C, 68.4; H, 8.7. Calc. for  $C_{20}H_{30}O_5$  : C, 68.5; H, 8.6%) reduces potassium permanganate solution in the cold and ammoniacal silver nitrate on gentle heating. When treated with sodium nitroprusside under conditions for the Legal test as modified by

\* It appears the tertiary hydroxy-group is eliminated when the hydrogenation is carried out in an acidic medium, three mols. of hydrogen being absorbed. Schwyzer *et al.* (*loc. cit.*) carried out the hydrogenation in neutral medium (ethanolic solution in presence of Adams's catalyst) but in this case total absorption represented two mols. and the product isolated was tetrahydroandrographolide,  $C_{20}H_{34}O_5$ , m. p. 180—188°. This has been confirmed by the present authors although the product had a higher m. p. The hydrogenation was carried out by shaking a solution of andrographolide (0.5 g.) and platinum oxide (50 mg.) in absolute ethanol (30 c.c.) in an atmosphere of pure hydrogen. Absorption, complete in 45 minutes, represented two mols. of hydrogen. After several crystallisations the product had m. p. 213—214° (Found : C, 68.0; H, 9.6. Calc. for  $C_{20}H_{34}O_5$  : C, 67.8; H, 9.6%).

Elderfield *et al.* (*loc. cit.*) it shows an intense purple-red colour which deepens and then fades. The ultra-violet absorption spectrum shows a maximum at 220  $\mu$ . ( $\log \epsilon = 4.1$  in absolute ethanol). On distillation with 33% sulphuric acid it does not split off formaldehyde.

*Triacetyl-andrographolide*.—A mixture of andrographolide (0.1 g.), freshly distilled acetic anhydride (0.5 c.c.), and a minute quantity of fused zinc chloride was heated to about 90° till a clear solution was obtained (about 5 minutes). The solution was cooled, treated with water (1 c.c.), and shaken vigorously. A heavy oil separated which gradually solidified. Triacetyl-andrographolide crystallises from ethanol in silky needles, m. p. 128° (yield, almost quantitative) (Found: C, 65.8; H, 7.4. Calc. for  $C_{26}H_{36}O_8$ : C, 65.5; H, 7.5%).

*Diacetyldeoxyandrographolide*.—A solution of triacetyl-andrographolide (0.6 g.) in ether was added to aluminium amalgam (prepared from 3.6 g. of aluminium foil) immersed in ether (300 c.c.). Water, in small quantities, was added to this mixture during about 3 hours, the end of the reduction being indicated when the product gave no red colour with methanolic potassium hydroxide. The ethereal solution was then washed with water and the ether evaporated off. The residue, on crystallisation from ethanol, separated as flat needles, m. p. 118–119° (yield, almost quantitative) (Found: C, 68.9; H, 8.1. Calc. for  $C_{24}H_{34}O_6$ : C, 68.9; H, 8.1%).

*Catalytic Hydrogenation of Andrographolide with Platinum in Acetic Acid*.—A solution of andrographolide (0.5 g.) in glacial acetic acid (25 c.c.) was shaken in an atmosphere of pure hydrogen with Adams's platinum oxide (50 mg.). Three mols. of hydrogen were smoothly absorbed in 10–15 minutes; absorption then ceased. The mixture was filtered and the filtrate kept in an evacuated desiccator over potassium hydroxide till four-fifths of the acetic acid was removed. The residual liquid was carefully diluted with water till just turbid. Colourless crystals slowly separated. On recrystallisation from dilute acetic acid these had m. p. 175°. They were dissolved in excess of acetic acid and the solution was diluted with water till just turbid. When this mixture was kept in the ice-chest for 2 days a small quantity of crystals separated, having m. p. 193°. Recrystallised twice from absolute ethanol (ice-chest) they gave glistening plates of the pure high-melting *deoxytetrahydroandrographolide*, m. p. 217° (Found: C, 70.7; H, 10.0.  $C_{20}H_{34}O_4$  requires C, 71.0; H, 10.0%).

The filtrate obtained after separation of the crystals, m. p. 193°, yielded a second crop when kept in the cold. After several crystallisations from ethanol containing a little water the lower-melting *deoxytetrahydroandrographolide* separated as microscopic needles, m. p. 141° (Found: C, 70.6; H, 9.8%).

*Catalytic Hydrogenation of Andrographolide in Methanol in Presence of Palladium*.—Andrographolide (0.4 g.) in methanol (40 c.c.) was shaken in an atmosphere of pure hydrogen in presence of 1% palladium chloride solution (2 c.c.) containing some free hydrochloric acid. The shaking was continued for about  $\frac{1}{2}$  hour after absorption was complete. After filtration the solution was concentrated under reduced pressure at room temperature until crystals separated. It was then left in the ice-chest overnight. The crystals, m. p. 205°, were then collected and recrystallised several times from absolute ethanol, giving the higher-melting *deoxytetrahydroandrographolide* as colourless glistening plates, m. p. 217° (Found: C, 70.7; H, 9.9.  $C_{20}H_{34}O_4$  requires C, 71.0; H, 10.0%).

The filtrate from the crystals of m. p. 205° was treated with water till turbid and left in the ice-chest, giving a second crop of crystals (m. p. 175°). These, after several crystallisations from ethanol, gave more of the higher-melting isomer and, from the mother-liquor, the lower-melting *isomer* which, crystallised from dilute ethanol, had m. p. 141° (Found: C, 70.8; H, 9.8%).

*Diacetyldeoxytetrahydroandrographolides*.—A solution of diacetyldeoxyandrographolide (0.3 g.) in glacial acetic acid (15 c.c.) was shaken in an atmosphere of pure hydrogen in presence of Adams's platinum oxide (30 mg.). Absorption (2 mols.) was complete in 10 minutes. After separation from the catalyst the clear solution was diluted with water till turbid. Minute crystals slowly separated which were collected and had m. p. 118° (shrinkage at 80–85°). After several crystallisations from ethanol containing a little water the higher-melting *diacetyldeoxytetrahydroandrographolide* was obtained in colourless prisms, m. p. 126° (opaque liquid) (Found: C, 68.3; H, 9.0.  $C_{24}H_{38}O_6$  requires C, 68.2; H, 9.0%).

The acid filtrate obtained after separation of the product of m. p. 118° was diluted with an equal volume of water; a second crop of crystals separated. It was collected and after several crystallisations from aqueous ethanol gave the lower-melting *isomer* as silky needles, m. p. 98° (Found: C, 68.0; H, 9.0%).

*Hydrolysis of Diacetyldeoxytetrahydroandrographolide, m. p. 126°*.—Diacetyldeoxytetrahydroandrographolide (50 mg.) of m. p. 126° was hydrolysed by a boiling solution of potassium

hydroxide (0.5 g. in water 1 c.c.) and ethanol (10 c.c.) for  $1\frac{1}{2}$  hours. Excess of ethanol was evaporated off and the residue was diluted with water. The solution was neutralised with dilute hydrochloric acid and the hydroxy-acid which separated was brought into solution by heat. The hot solution was treated with a few drops of concentrated hydrochloric acid. Minute needle-shaped crystals slowly separated in the cold. Crystallised from ethanol containing a little water, these had m. p.  $141^\circ$  alone or mixed with the lower-melting deoxytetrahydroandrographolide (Found: C, 70.9; H, 10.0%).

*Hydrolysis of Diacetyldeoxytetrahydroandrographolide, m. p.  $98^\circ$ .*—Hydrolysis of the lower-melting diacetyldeoxytetrahydroandrographolide (50 mg.) was carried out as in the preceding experiment. The product obtained on acidification and lactonisation was crystallised from ethanol, giving shining plates of the deoxytetrahydroandrographolide, m. p. and mixed m. p.  $217^\circ$  (Found: C, 70.8; H, 9.9%).

*Catalytic Hydrogenation of Triacetylandrographolide.*—Triacetylandrographolide (0.2 g.) in glacial acetic acid (10 c.c.) was shaken in an atmosphere of pure hydrogen in presence of platinum oxide (20 mg.). The absorption (3 mols.) was complete in 10–15 minutes. As the hydrogenated product was difficult to isolate it was directly deacetylated as in the previous experiment. The product obtained in this way had m. p.  $175^\circ$  (Found: C, 70.6; H, 9.7%) and appeared to be a mixture of the two isomers of deoxytetrahydroandrographolides. It did not give a red colour with potassium hydroxide in methanol. The isomers were not separated.

*Deoxytetrahydroandrographolic Acid.*—The mixed isomers of deoxytetrahydroandrographolide (m. p.  $175^\circ$ ) (0.1 g.) obtained above were heated under reflux for an hour with ethanol (1.5 c.c.), water (0.5 c.c.), and potassium hydroxide (0.2 g.). The solution was then treated with some more ethanol and a small amount of charcoal, heated for a short while, and filtered. After removal of excess of ethanol the product was diluted with water, cooled in ice, and acidified to Congo-red with very dilute hydrochloric acid. A flocculent precipitate separated which was immediately filtered off and washed well with ice-cold water. *Deoxytetrahydroandrographolic acid* obtained in this way crystallised from water in silky needles, m. p.  $107$ – $108^\circ$ . It was dried at room temperature *in vacuo* (Found: C, 65.9; H, 10.1.  $C_{20}H_{36}O_5, \frac{1}{2}H_2O$  requires C, 65.7; H, 10.1%).

*Ozonolysis of Andrographolide.*—Ozonised air was passed for 45 minutes through a solution of andrographolide (0.2 g.) in glacial acetic acid (10 c.c.). The product was diluted with an equal volume of water and distilled in steam. The distillate was collected directly in a saturated solution of 2:4-dinitrophenylhydrazine in 2N-sulphuric acid. The 2:4-dinitrophenylhydrazone (0.12 g.) obtained was crystallised from ethanol, separating in yellow silky needles, m. p.  $166^\circ$  alone or mixed with that of formaldehyde.

The solution remaining after steam-distillation was treated with a solution of 2:4-dinitrophenylhydrazine in 2N-sulphuric acid. An orange-yellow precipitate was obtained. This was dissolved in ethanol and the solution allowed to crystallise. An extremely small amount of a red crystalline product, m. p.  $248^\circ$  (decomp.), was obtained.

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DEPARTMENT OF CHEMISTRY, BETHUNE COLLEGE, CALCUTTA.  
DEPARTMENT OF CHEMISTRY, SCHOOL OF TROPICAL MEDICINE,  
CALCUTTA.

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