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INVESTIGATIONS ON PLANTS OF WEST AFRICA—II

ISOLATION OF ANTHOCLEISTIN FROM *ANTHOCLEISTA PROCERA* (LOGANIANCEAE)

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Abstract—Anthocleistin, a new triterpene has been isolated and characterized. The functional groups have been determined and several derivatives prepared. The various spectroscopic data are given and interpreted.

THE use of native plants in West African tribal societies has been known for centuries and has recently been receiving increasing scientific attention. The investigations of these plants is to determine their possible values and to study the structures of their constituents. *Anthocleista procera* Afzel is a tree of the Loganiaceae family known in West Africa as cabbage tree. In Sierra Leone the leaf is used for abdominal pains of uterine origin.[2]

The fruits of the plant were dried and powdered. From the basified powder an alkaloid was isolated which was identified as gentianine, and its complete characterization has already been reported.[3]

Soxhlet extraction of the powdered non-basified material with n-hexane and chloroform yielded from the solution a crystalline product which on chromatoplate was resolved into two spots. Chromatography on acid washed alumina gave a pure substance which was named anthocleistin. The substance decolourized a solution of potassium permanganate, gave a yellow colour with tetranitromethane and a positive test with Liebermann-Burchard reagent (reddish–purple colour) indicating unsaturation. In spite of the fact that a positive test was obtained with Tollens' reagent, neither an α-hydroxy ketone nor an aldehyde function was found to be present. The compound which analysed for $C_{30}H_{46}O_3$, had the following characteristics: sublimation range 240–250°, $[\alpha]_D$ +51°; its IR spectrum showed a broad absorption in the hydroxyl region and a band at 833 cm^{-1} was related to a trisubstituted double bond, an observation which was supported by an end absorption in the UV spectrum.

Most of the reactions for the characterization of anthocleistin were done with its monoacetate which was purified by chromatography and repeated crystallization. The monoacetate analysed for $C_{32}H_{48}O_4$ and sublimed over the range of 210–230°, $[\alpha]_D$ +81°, the IR spectrum showed the absorption bands at 1705 cm^{-1}, 1750 cm^{-1} for a carbonyl and for acetoxy carbonyl, a broad band between 3500 and 3000 cm^{-1} for an hydroxyl group, which in conjunction with that at 1705 cm^{-1} could be interpreted as

[1] A part of this work was carried out at the Daniel Sieff Research Institute, The Weizmann Institute of Science Rehovoth, Israel during the tenure of the Rev. Solomon B. Caulkner Memorial Fellowship 1962–63.

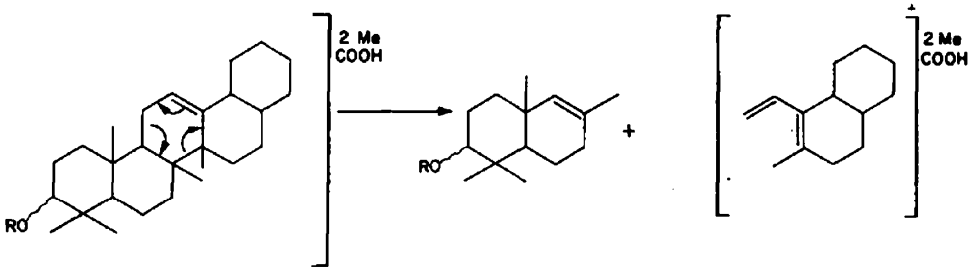
[2] J. M. Dalziel, *The Useful Plants of West Tropical Africa* 2nd reprint p. 361. Crown Agents for Overseas Governments and Administrations, London (1955).

[3] D. Lavie and R. Taylor-Smith, *Chem. & Ind.* 781 Part I: (1963).

due to a carboxylic acid. The strong band at 1238 cm^{-1} is however related to that at 1750 cm^{-1} for the acetoxy group. The NMR spectrum indicated a set of several peaks at high field for at least six methyl groups at the following values $\tau = 9{\cdot}20$, 9·12 ($2XCH_3$) 9·07 ($2XCH_3$) and 8·90. A sharp peak at $\tau = 7{\cdot}94$ was related to the three protons of the acetoxy group, while at low field a triplet centered at $\tau = 5{\cdot}44$ was assigned to the proton next to the acetoxy group. The broad and unresolved signal at $\tau = 4{\cdot}70$ is due to the vinylic proton of the trisubstituted double bond. The integration of the total spectrum accounted for about 50 protons.

The mass spectrum of this mono-acetate derivative showed the mass peak at 498 with other peaks at 438, corresponding to loss of acetic acid from the molecule, peaks at 483, and 423 correspond to loss of methyl from the parent compound, and from the compound with mass peak at 438.

An interesting feature of the spectrum is the base peak at 248 which is one less than half of the mass peak. This suggests that the molecule splits into two equal halves by rearrangement with a proton. At low mass many peaks differing by 14 were obtained and this is consistent with loss of angular methyls a possible structure consistent with the above data.



This is consistent with an α or β amyrin derivative with a carboxyl group on rings D or E.[4] Such fragmentations as above are known to occur by a retro-diels-alder reaction involving ring C as shown with the charge remaining on the diene fragment.[4,5] Retro diels alder fragmentation also leads to the location of the double bond at the 12–13 position as pointed out by Djerassi *et al.*

Optical rotatory dispersion measurements show the absence of a ketonic carbonyl group and a cotton effect below 250 mμ which could be due to an acetate.[6]

Further evidence for the carboxylic acid group was obtained by the formation of the sodium salt with alcoholic sodium hydroxide, and by methylation of anthocleistin with diazomethane. This gave a crystalline compound of the methyl ester which analysed for $C_{31}H_{50}O_3$—CH_3OH (1 mole methanol of crystallizations) $[\alpha]_D$ +40, m.p. 116°). The IR spectra showed bands at 3500 cm^{-1} for an hydroxyl group and at 1745 cm^{-1} for an ester carbonyl. Anthocleistin acetate was next methylated with diazomethane and white crystalline needles were isolated this analysed for $C_{33}H_{50}O_4$ with $[\alpha]_D$ +58·5 and m.p. 203–204°. Its IR spectra showed a sharp band for the carbonyl

[4] H. Budzikiewiez, J. M. Wilson and C. Djerassi, *J. Amer. Chem. Soc.* **85**, 368 (1963).
[5] F. H. Field and J. H. Franklin, *Electron Impact Phenomena* p. 92. Academic Press, New York N.Y. (1957).
[6] Private communication from Prof. C. Djerassi.

group at 1745 cm^{-1} and a sharp band at 1242 cm^{-1} for the acetoxyl group and no absorption for hydroxyl.

Oxidation of this compound with chromic acid gave an α,β-unsaturated compound which showed two sharp bands at 1695 cm^{-1} and 1745 cm^{-1} and on absorption in the UV at λ_{max} 250 mμ ε = 13,320. It analysed for $C_{33}H_{48}O_5$ $[\alpha]_D$ +33·3, m.p. 232–233°. Similar characteristics are known for triterpenes with α-amyrin type nucleus.[7,8]

Oxidation of the alcohol group in anthocleistin with chromium trioxide in acetone at 0° gave a product showing only a slight increase of carbonyl absorption at ν_{max} 1701 cm^{-1} which proved to be identical with the starting material. However, when the monoacetate was oxidized with the same reagents at about 40° a new crystalline compound possessing the characteristics of an α,β-unsaturated ketone was obtained: ν_{max} 1669 for the carbonyl and 1637 cm^{-1} for the conjugated double bond; λ_{max} 251 mμ ε = 11, 540; NMR showed a singlet at τ = 4·20. The fact that the broad band (at τ = 4·70) referred above became a sharp singlet in the oxidation product clearly indicates that the vicinal position of the vinylic proton is now occupied by the newly introduced oxygen function. This effect is also observed to cause a displacement of the protons of at least two methyl groups to lower field. Reduction of anthocleistin acetate with LAH resulted in the formation of a diol which upon acetylation gave a diacetate. This product analysed for $C_{35}H_{58}O_4$, with 1 molecule methanol of crystallization, displayed in NMR spectrum a strong sharp peak at τ = 6·96 accounting unequivocally for the six protons of two acetoxy groups. The triplet at τ = 5·50 already encountered in the monoacetate for the proton adjacent to the acetoxy group was present, and furthermore two doublets of an AB type[9] centered respectively at τ = 5·86 and 6·34 (J = 11 c/s) indicated that the second acetoxy group in the molecule resulted from acetylation of a primary alcohol group. The latter under the conditions of the reaction arises in anthocleistin from the conversion of its carboxylic group to a primary alcoholic group by reduction.

EXPERIMENTAL

M.p. were taken on a Micro Koffler hot-stage microscope. All optical rotation measurements were carried out in EtOH. UV absorption spectra were done on a Cary 14 Spectrophotometer and a Bausch and Lomb Spectronic 505 in EtOH. IR spectra were recorded on a Perkin-Elmer Infracord model 137 spectrometer equipped with a NaCl prism and, unless otherwise stated, were determined in $CHCl_3$ solution in 5–10% concentration. NMR spectra were recorded on a Varian A-60 Spectrometer, the spectra were determined in $CDCl_3$ of about 5–10% concentration containing tetramethylsilane as internal standard. Thin layer chromatography was done on chromatoplates of silica gel G (Merck) and spots were developed with $KMuO_4$ 0·5% solution in a saturated cupric acetate solution.

Extraction of gentianine. To 1 kg of powdered fruits of *Anthocleista procera* in a 5 l. conical flask, 315 ml 2 N NH_4OH was added gradually with periodic agitation. The powder was allowed to soak for 24 hr, then 2 l. $CACl_3$ were added and the whole stirred continuously for 24 hr. The mixture was then filtered and the residue having been washed with $CACl_3$ and pressed dry in a Buchner funnel, was transferred to the 5 l. conical flask. The extraction was repeated several times with the same solvent, until tests with acidified extracts were negative with the usual alkaloidal reagents.

The combined $CACl_3$-extracts were concentrated by evaporation to a small volume and the alkaloid extracted with a 5% HCl aq. The acid extract was neutralized with Na_2CO_3 aq and extracted with ether. The organic layer was washed with water, dried over Na_2SO_4, filtered, and evaporated to

[7] J. S. Spring and T. Vicker-Staff, *J. Chem. Soc.* 1859 (1934).
[8] D. H. R. Barton and N. J. Holness, *J. Chem. Soc.* 78 (1952).
[9] L. M. Jackman, *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry* p. 89. Pergamon Press, London (1959).

yield 6·58 g of a white crystalline substance. Several recrystallizations from benzene–pet. ether gave white needles of gentianine, m.p. 81–82°; all spectroscopic data for this substance have been previously reported.[8] (Found C, 68·64; H, 5·47; N, 7·85 $C_{10}H_9O_2N$ requires: C, 68·56; H, 5·18; N, 8·02%.) The hydrochloride was prepared in anhydrous conditions m.p. 169–170°.

Dihydrogentianine. Gentianine (61 mg) dissolved in abs. EtOH was hydrogenated over Pd–C (9 mg). When the calculated amount of H_2 was absorbed (8·2 ml) the reaction was discontinued and the solution filtered free from catalyst. Evaporation of the solvent to dryness left a residue which crystallized from benzene–pet. ether, m.p. 75–76°, λ_{infl} 225 mμ (ε = 4,450) and 272 mμ (ε = 1,550); in the IR the band at 1626 cm^{-1} present in gentianine had disappeared. (Found: C, 67·73; H, 6·15; N, 7·84. $C_{10}H_{11}O_2N$ requires: C, 67·78; H, 6·26; N, 7·91%.)

Isolation of anthocleistin. Dry powdered fruits of *Anthocleista procera* (1,500 g) were extracted in a soxhlet with 6 l. n-hexane for 4 days. The solvent was then changed to $CHCl_3$, 6 l. of solvent being used for the extraction which lasted 4 days. Upon standing a waxy cream solid crystallized from the solution. By decantation and filtration, 3·74 g of solid were collected. Upon concentration of the filtrate, an additional 5·48 g of the same substance was isolated. The same procedure was repeated with two batches of 1,500 g each and the respective weights of the substance isolated were 10·42 g and 11·81 g. Thus from a total weight of 4,500 g of dry powdered fruit 31·45 g of crude crystalline material was isolated, yield 4·7% of crude solid. The solid was dissolved in MeOH, decolourized with charcoal and diluted with water to yield a white crystalline substance which showed two spots on chromatoplate (developed with benzene–ethyl acetate 3:1). This substance (243 mg) in $CHCl_3$ was chromatographed on a column of acid-washed alumina (40 g), using benzene and benzene–chloroform (1:1) as eluents. The crystalline substance emerging with the latter solvent mixture showed only one spot on chromatoplate. Several of these fractions were combined and crystallized from MeOH yielding needles (187 mg); sublimation range 210–240°, $[\alpha]_D$ +51° (*c* 0·9); ν_{max}^{KBr} 1700, 1645, 1458, 1391, 1377, 1047, 1029, 997 and 833 cm^{-1}. UV end absorption (Found: C, 78·69; H, 10·64 $C_{30}H_{46}O_3$ requires: C, 79·24; H, 10·20%.)

Anthocleistin acetate. Anthocleistin (870 mg) was acetylated over night at room temp in a mixture of dry pyridine (12 ml) and acetic anhydride (12 ml). The mixture was decomposed and the solid collected by sublimation 210–230°, $[\alpha]_D$ +81° (*c*. 0·99); ν_{max} 1733 and 1242 cm^{-1} for the acetoxy group. (Found: C, 76·82; H, 9·98 $C_{32}H_{48}O_4$ requires: C, 77·37 H, 9·74%.)

The crude acetate (850 mg) dissolved in benzene was chromatographed on acid washed alumina (40 g). The column was eluted with the following solvents in succession: n-pentane, pentane–benzene (2:1), benzene and chloroform. The bulk of the substance (530 mg) was obtained from the last solvent. It showed one spot on chromatoplate (developed with benzene–ethyl acetate 2:1) and upon crystallization with MeOH yielded pure anthocleistin acetate). Pure anthocleistin acetate has also been obtained by repeated recrystallizations from MeOH.

Oxidation of anthocleistin acetate. To a solution of anthocleistin acetate (250 mg) in acetone (10 ml; previously distilled over $KMnO_4$), a solution of CrO_3 (27 g CrO_3 in 100 ml of 35% H_2SO_4 aq) was added dropwise. When 1·5 ml of the oxidizing mixture was consumed, the brown-red colour persisted, and the temp reached about 45°. The mixture was left stirred for 3 hr at room temp. Excess oxidant was then reduced with MeOH and the solution concentrated to half its volume. Following the addition of water the mixture was shaken with $CHCl_3$. The organic layer was separated and dried over Na_2SO_4. By evaporation of the solvent a crystalline product was obtained (240 mg); recrystallizations from boiling MeOH, yielded needles which sublimed with dec. 285°, $[\alpha]_D$ +71° (*c*. 0·97); ν_{max}^{KBr} 1736, 1709, 1669 and 1637 cm^{-1}. (Found: C, 74·68; H, 9·32. $C_{32}H_{46}O_5$ required: C, 75·26: H, 9·08%.)

Methyl ester of anthocleistin acetate. Anthocleistin acetate (959 mg) was dissolved in MeOH and cooled in ice. Excess diazomethane was then added dropwise, and the excess later destroyed with acetic acid and diluted with $CHCl_3$. The solution was washed with $NaHCO_3$ aq, and later with water, and then dried with Na_2SO_4. After filtration and evaporation white crystalline needles 705 mg m.p. 203–204°, $[\alpha]_D$ +58·5 (*c*, 0·8) was obtained; ν_{max} 1745 cm^{-1} and 1242 cm^{-1}. (Found: C, 77·51; H, 10·10. $C_{33}H_{50}O_4$ required: C, 77·64; H, 9·80%.)

Oxidation of Methyl ester of anthocleistin acetate. The above methyl ester (280·5 mg) was dissolved in acetone (10 ml, previously distilled over $KMnO_4$), a solution of CrO_3 (27 g of CrO_3 in 100 ml of 35% H_2SO_4 aq) was added dropwise to a slight excess. The mixture was left stirred for 3 hr at room temp. Excess oxidant was then reduced with MeOH and the solution was concentrated to half

its volume. Following the addition of water the mixture was shaken with $CHCl_3$. The organic layer was separated and dried with Na_2SO_4. By evaporation of the solvent a crystalline product was obtained (150 mg); recrystallizations from MeOH gave needles m.p. 232–233, $[\alpha]_D$ +33·3 (*c*. 0·76). ν_{max}^{KBr} 1635, 1695, 1745 cm^{-1}; λ_{max} 250 mμ ε = 13,320. (Found C, 75·07; H, 9·67 $C_{33}H_{44}O_6$ required: C, 77·64; H, 9·80%.)

Methyl ester of anthocleistin. An ice cold solution of anthocleistin (50 mg) in MeOH was treated with an excess of a solution of diazomethane in ether. Excess reagent was destroyed with acetic acid and diluted with $CHCl_3$. The solution was washed with $NaHCO_3$ aq and water and then dried over Na_2SO_4. Evaporation of the solvent left an oil which crystallized from MeOH; m.p. 116°, $[\alpha]$- + 40° (*c*, 1·01); ν_{max} 1721 cm^{-1}. (Found: C, 77·17; H, 10·29. $C_{31}H_{50}O_3 \cdot CH_3OH$ or $C_{31}H_{54}O_4$ requires: C, 76·50; H, 10·36%.)

Lithium aluminium hydride reduction of anthocleistin acetate to the diol. To a suspension of LAH (770 mg) in tetrahydrofuran (150 ml), a solution of anthocleistin acetate (528 mg) in the same solvent (50 ml) was added. The mixture was heated under reflux with stirring for 4 hr, then decomposed by adding ethyl acetate (5 ml), and sat. Na_2SO_4 aq. The solution was filtered from the insoluble salts, which were washed exhaustively with $CHCl_3$. The combined filtrates were dried over Na_2SO_4 and evaporated to dryness. The remaining solid crystallized from MeOH as platelets (459 mg), it showed only one spot on chromatoplate; m.p. 223–224°; $[\alpha]_D$ +65° (*c*, 0·65); 3584 cm^{-1} (v. strong); UV end absorption. (Found: C, 78·41; H, 11·27. $C_{30}H_{50}O_2$ CH_3OH or $C_{31}H_{54}O_3$ requires: C, 78·55; H, 10·99%.)

The diacetate was prepared by acetylation of the above driol in a solution of acetic anhydride and pyridine and allowed to stand cover night at room temp. The product (165 mg) crystallized from MeOH in rosette like clusters of needles; m.p. 158–160°, $[\alpha]_D$ +49° (*c*, 0·92); ν_{max} 3704, 1727 and 1248 cm^{-1}. (Found: C, 75·39; H, 10·12. $C_{34}H_{54}O_4 \cdot CH_3OH$ or $C_{35}H_{58}O_5$ requires: C, 75·23; H, 10·03%.)

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