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CHEMISTRY OF *AEGICERAS MAJUS* GAERTN—V

STRUCTURE OF THE TRITERPENE AEGICERIN

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Abstract—The structure of aegicerin, $C_{30}H_{48}O_3$, a new triterpene from *Aegiceras majus* Gaertn is defined as 3β-hydroxy-13β:28-epoxy-16-oxo-18β-oleanane (Ia).

IN AN earlier communication[1] the isolation of a new triterpene sapogenin, aegicerin, from the bark of *Aegiceras majus* Gaertn was described. Aegicerin was also encountered in the non-glycosidic extract of the bark from which it was isolated[2] with the sterols after saponification. Herein are reported studies leading to the structure of this compound.

Aegicerin (Ia), m.p. 254–256°, $[\alpha]_D^{28}$ −23·6°, is a neutral triterpene of elementary composition $C_{30}H_{48}O_3$. It forms a monoacetate (Ib) on mild acetylation and an oxime under forcing conditions which characterized two of the oxygen functions as primary or equatorially oriented secondary hydroxyl and carbonyl groups (ν_{max} in $CHCl_3$ at 3550 and 1695 cm^{-1}). With acetic anhydride–sodium acetate, aegicerin oxime furnishes an oxime diacetate, thus supporting the classification of the carbonyl as the keto group. Confirmation was obtained when aegicerin acetate was recovered unchanged on chromic acid oxidation.

On oxidation with chromium trioxide in pyridine[3] or chromic acid-sulphuric acid in acetone[4] aegicerin yields a colorless diketone, $C_{30}H_{46}O_3$, ketoaegicerin (Ic) which is stable to base and gives a positive Zimmermann test[5] for a 3-keto group (with aegicerin the test is negative). Reduction of aegicerin with sodium borohydride gives a triol, $C_{30}H_{50}O_3$, which together with its triacetate (IIb) was identified as "genin-A" (3β,16α,28-trihydroxy-oleana-12-ene, IIa) by direct comparison (mixed m.p.) with an authentic sample.[6] Thus aegicerin might have a β-amyrin skeleton with the hindered keto group at C_{16}. The optical rotatory dispersion curve[7] of aegicerin acetate shows a negative Cotton effect (with resolution of the trough) and is consistent[8] with a 16-keto function.

The possibility that aegicerin could be 16-deoxy-16-oxo genin-A (IIIa) is excluded as it forms only a monoacetate stable to chromic acid oxidation and on treatment with alkali it is recovered unchanged without undergoing retro-aldolization to

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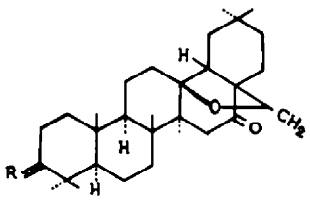
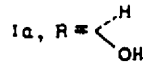
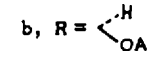


Ia, R = 

b, R = 

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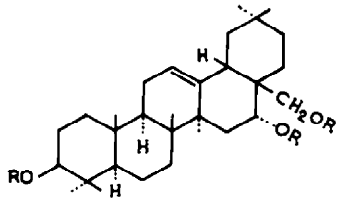

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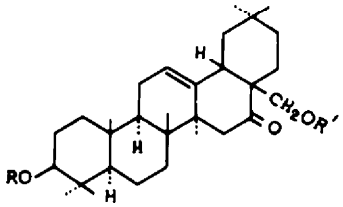
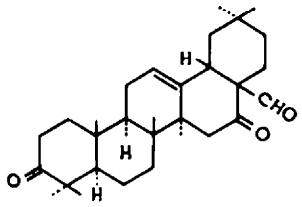


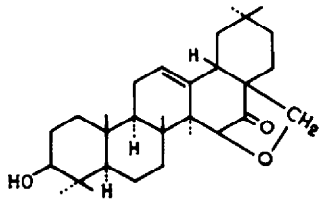
IIIa, R = R′ = H

b, R = R′ = Ac

c, R = Ac; R′ = H



IV



V

norechinocystenolone[9] as could be expected[5] of structure IIIa. Further, ketoaegicerin then becomes an alkali-labile[10] formyl ketone represented by IV which should yield with a base the known norechinocystenedione.[9] But ketoaegicerin is not alkali-sensitive.

Aegicerin and its derivatives possess an inert oxygen atom regarded as oxidic. Aegicerin exhibits IR bands (in $CHCl_3$ solution) at 1120, 1075 and 1040 cm^{-1} suggesting[11] the presence of a C—O—C group. This was confirmed by the conversion of aegicerin acetate (Ib) with acetic anhydride and tolune-*p*-sulphonic acid[12] into a diacetate, identified by direct comparison with an authentic sample,[1] as 3β,28-diacetoxy-16-oxo-oleana-12-ene (IIIb) and another compound, m.p. 219–220°, which might be IIIc. The oxide bridge in aegicerin should originate from C_{28} and models indicate that it could be joined to C_{15}, C_{19} or C_{21} of a β-amyrene or C_{13} of an oleanane skeleton.

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The formation of IIIb from aegicerin could only be explained[13] if the oxide bridge in the latter terminates on C_{13} of an oleanane skeleton, thus favouring structure Ia for it. In the sodium borohydride reduction (*vide supra*) of aegicerin to "genin-A" (IIa) opening up of the oxide bridge with the introduction of a double bond at its terminating point takes place as in the reaction with toluene-*p*-sulphonic acid and acetic anhydride. This could again result by an elimination reaction under the acid conditions used in the working up of the product (*vide experimental*).

In agreement with the above formulation (Ia) aegicerin gives no colour with tetranitromethane for unsaturation and its acetate is stable to chromic acid oxidation under conditions when β-amyrin acetate forms an $\alpha\beta$-unsaturated ketone.

The possibility of aegicerin being a limonilic-type cyclization product[14] of 16-deoxy-16-oxo genin-A (IIIa) seemed an attractive hypothesis especially because of its occurrence in the same plant with genin-A and structure (V) was assigned[15] to it. This is untenable as the α-keto oxide moiety in (V) should by analogy with 24-nor-22-oxo-16α:21α-oxido-oleana-12-ene, an aescigenin derivative[12b], be unaffected at the oxide ring by sodium borohydride. The opening of the oxide bridge in aegicerin acetate with acetic anhydride and toluene-*p*-sulphonic acid cannot give rise to 16-oxo erythrodiol diacetate (IIIb) without the formation of a double bond. Further aegicerin and its derivatives do not have, in their IR spectra[16], absorption bands due to the additional 5-membered ring ketone involving the oxide function as would be expected[11,12b] of structure (V).

The 60 mc nuclear magnetic resonance spectrum[17] of aegicerin which discloses no signal for vinyl proton supports structure Ia for it. The NMR spectrum shows a multiplet corresponding to one proton and centred at 6·27τ for one of the 28-H on carbon bearing the oxide bridge and another complex multiplet centred at 6·81τ and corresponding to two protons for the second 28-H and for 3α(axial)-H on carbon having the hydroxyl group. A doublet at 7·24τ and 7·47τ with a peak area of one proton could be due to -OH and a signal at 7·85τ might be ascribed to the α-keto methylene group.

EXPERIMENTAL*

Aegicerin acetate (Ib)

Aegicerin (Ia, 1g) was treated overnight at room temp with pyridine (10 ml) and acetic anhydride (8 ml) and a benzene solution of the product passed through a column of alumina (60 g). Elution with benzene–light petroleum (1:1; 500 ml) gave aegicerin acetate (Ib, 900 mg) crystallizing from

* All m.ps were taken in a $KHSO_4$ bath and are uncorrected. Elementary analyses were performed by Drs. Weiler and Strauss, Oxford, England. Samples for analysis were dried *in vacuo* over P_2O_5 at 110° for 6 hr. Optical rotations were determined in $CHCl_3$ at room temp. Light petroleum refers to fraction b.p. 60–80°, and alumina used for chromatography is of Brockmann's (E. Merck) grade.

[13] The author is grateful to Dr. T. G. Halsall, Dyson Perrins Laboratory, Oxford, England and Dr. P. de Mayo, University of Western Ontario, London, Canada for the suggestion.

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[15] K. V. Rao, *Chem. & Ind.* 1523 (1963).

[16] Thanks are due to Prof. Carl Djerassi, Stanford University, Stanford, and to Dr. S. K. Chakraborti, Buffalo University, Buffalo, U.S.A., for IR spectra.

[17] The author is grateful to Dr. C. F. Hammer, Brandeis University, Waltham, U.S.A., for the NMR spectrum taken in CCl_4 solution with tetramethylsilane as an internal reference on a Varian A60 spectrometer.

chloroform–methanol as colorless flakes, m.p. 273–275°, $[\alpha]_D^{30}$ −17·7° (*c*, 1·07), $\nu_{max}^{CHCl_3}$ 1695 cm^{-1} (CO), 1715 and 1245 cm^{-1} (OAc), R.D. in dioxane (*c*, 0·20), $[\alpha]_{321}$ −1560°, $[\alpha]_{313}$ −1020°, $[\alpha]_{311}$ −1080°, $[\alpha]_{281}$ +1870°, (Found[18]: C, 77·27; H, 9·99; CH_3CO, 9·51. $C_{32}H_{50}O_4$ requires: C, 77·10; H, 10·04; CH_3CO, 8·63%).

Aegicerin (Ia)

Aegicerin acetate (Ib, 400 mg) was refluxed with alcoholic KOH (10%; 25 ml) for 8 hr. Dilution with water and isolation by means of ether gave a product which was chromatographed in benzene solution over alumina (40 g). Elution with benzene (500 ml) furnished aegicerin (Ia, 275 mg) crystalizing from methanol as microcrystalline needles, m.p. 254–256°, $[\alpha]_D^{28}$ −23·6° (*c*, 0·87), $\nu_{max}^{CHCl_3}$ 3550 cm^{-1} (OH), 1695 cm^{-1} (CO), 1120, 1075 and 1040 cm^{-1} (C—O—C), 1020 and 985 cm^{-1} (C—O—). (Found: C, 78·60; H, 10·48. $C_{30}H_{48}O_3$ requires: C, 78·94; H, 10·52%). It gave no perceptible colour with tetranitromethane and exhibited no characteristic high intensity UV absorption maximum in ethanol. Aegicerin develops a pink colour in the Liebermann-Burchard test and no colour in the Zimmermann reaction. The compound was recovered unchanged on oximation with hydroxylamine hydrochloride and pyridine overnight at room temp and on attempted formation of 2,4-dinitrophenylhydrazone under the usual conditions.

On reacetylation with acetic anhydride and pyridine (3 ml each) on the steam bath for 3 hr and subsequent setting aside at room temp for 48 hr aegicerin (100 mg) formed only the monoacetate (Ib) identified by mixed m.p. and optical rotation.

Aegicerin oxime

Aegicerin (Ia, 200 mg) in pyridine (3 ml) containing hydroxylamine hydrochloride (150 mg) was heated on the steam-bath for 12 hr and then left at room temp for 24 hr. The reaction mixture was poured into crushed ice and the collected solid in benzene was adsorbed on a column of alumina (20 g). Elution with ether–benzene (1:1; 300 ml) yielded aegicerin oxime (150 mg) crystallizing from aq. ethanol as microcrystalline needles, m.p. 244–246°, $[\alpha]_D^{26}$ −6·9° (*c*, 0·73), ν_{max}^{KBr} 3550 cm^{-1} (OH), 3350 cm^{-1} (N—OH), 1635 cm^{-1} (C=N—). (Found: C, 76·37; H, 10·33; N, 3·02. $C_{30}H_{49}O_3N$ requires: C, 76·43; H, 10·40; N, 2·97%.

The oxime (50 mg) was heated on the steam-bath for 3 hr with acetic anhydride (2 ml) containing fused sodium acetate (100 mg). Crystallization from ethanol gave aegicerin oxime diacetate (35 mg) m.p. 210–211°, ν_{max}^{KBr} 1755 and 1200 cm^{-1} (=N—OAc), 1720 and 1235 cm^{-1} (OAc) and 1625 cm^{-1} (C=N—). (Found: C, 74·18; H, 9·7; N, 2·72. $C_{34}H_{53}O_5N$ requires: C, 73·51; H, 9·54; N, 2·52%).

Ketoaegicerin (Ic)

(a) *Oxidation of aegicerin* (Ia) *with chromium trioxide-pyridine.* Aegicerin (Ia, 200 mg) in pyridine (5 ml) was treated at 0° with a suspension of CrO_3 (200 mg) in pyridine (10 ml) and the mixture was left at room temp for 14 hr. After adding much water, the material was extracted with ether which was washed well with dil. HCl, dried and evaporated. Trituration with ethanol yielded a solid (170 mg) which after chromatography over alumina, furnished ketoaegicerin (Ic) as colorless needles from chloroform–methanol m.p. 258–260°, $[\alpha]_D^{24}$ −2° (*c*, 0·98), ν_{max}^{KBr} 1702 cm^{-1} (CO): 1120, 1080, 1040, 1010, 990 and 885 cm^{-1}. (Found: C, 79·76; H, 10·30. $C_{30}H_{46}O_3$ requires, C, 79·29; H, 10·13%). It developed a pink color in the Zimmermann test with *m*-dinitrobenzene and alcoholic KOH.

(b) *Oxidation of aegicerin* (Ia) *with chromic acid-sulphuric acid.* Aegicerin (Ia, 100 mg) in "AnalaR" acetone (10 ml) was left with CrO_3–H_2SO_4 reagent[4] (1 ml) at room temp for 2 hr. After dilution with water and destruction of excess of the oxidant with methanol, the product (only a neutral fraction) crystallized from ethanol giving ketoaegicerin, as needles, m.p. and mixed m.p. 258–260°.

Ketoaegicerin (Ic, 100 mg) was heated under reflux for 6 hr with alcoholic KOH (5%; 20 ml). Ether extraction recovered the starting material identified by m.p. and mixed m.p. 253–255°.

Oxime of ketoaegicerin

Ketoaegicerin (Ic, 50 mg) and hydroxylamine hydrochloride (100 mg) in pyridine (5 ml) was left at room temp overnight. Crystallization of the product from chloroform–methanol gave the

[18] By the earlier erroneous analysis (ref 2) it was considered a diacetate.

mono-oxime of ketoaegicerin, m.p. 260–263°. (Found: 76·43; H, 9·86; N, 3·06. $C_{30}H_{47}O_8N$ requires: C, 76·75; H, 10·02; N, 2·99%).

When the above reaction mixture was heated on the steam-bath for 6 hr and then left at room temp for 48 hr, the dioxime of ketoaegicerin was obtained, crystallizing as needles from ethanol m.p. 277–278°. (Found: C 74·27; H, 9·72; N, 6·04. $C_{30}H_{48}O_8N_2$ requires: C, 74·38; H, 9·91; N, 5·78%).

Chromic acid oxidation of aegicerin acetate (Ib)

Treatment of aegicerin acetate (Ib, 220 mg) in glacial acetic acid (25 ml) with CrO_3 (100 mg) in the same solvent (8 ml) at room temp afforded the unreacted material (190 mg) crystallizing from ethanol, m.p. and mixed m.p. 270–273°.

In another experiment aegicerin acetate (150 mg) in glacial acetic acid (10 ml) was oxidized with CrO_3 (100 mg) in the same solvent (6 ml) on the steam-bath for 3 hr. After dilution with water the excess of chromic acid was destroyed and the product was separated into acidic and neutral fractions. Crystallization of the former from aqueous ethanol gave microcrystalline needles (20 mg), m.p. 273°. (Found: C, 71·85; H, 9·10%). The acid showed no selective absorption in the UV above 210 mμ. The neutral fraction (75 mg) recovered was aegicerin acetate.

Sodium borohydride reduction of aegicerin (Ia)

Aegicerin (Ia, 300 mg) in methanol (30 ml) was treated at room temp for 15 hr with $NaBH_4$ (300 mg). After adding HCl (3 ml) and water (20 ml) the reaction mixture was warmed on the steam-bath for 10 min and then diluted with water. The collected solid was purified by chromatography in chloroform–benzene (1:4) solution over alumina (25 g). Crystallization of the chloroform–benzene (1:1; 400 ml) eluates from benzene–ether furnished needles (210 mg), m.p. 240–242°, $[\alpha]_D^{26}$ +43·1° (*c*, 0·43), identified as "genin-A" (IIa) by mixed m.p. (Found: C, 78·24; H, 11·17. $C_{30}H_{50}O_8$ requires: C, 78·60; H, 10·91%).

The above reduction product (IIa, 100 mg) was acetylated with acetic anhydride and pyridine (3 ml each) on the steam-bath for 3 hr and then at room temp for 48 hr. The product crystallized from methanol, yielding "genin-A triacetate" (IIb, 85 mg) as needles m.p. and mixed m.p. 158–160°, $[\alpha]_D^{26}$ −9° (*c*, 0·55). (Found: C, 74·40; H, 9·63. $C_{36}H_{56}O_8$ requires: C, 73·97; H, 9·58%).

*Acetic anhydride and toluene-*p*-sulphonic acid treatment of aegicerin acetate* (Ib)

Aegicerin acetate (150 mg) in refluxing acetic anhydride (5 ml) was treated with toluene-*p*-sulphonic acid (70 mg) and refluxed for 1½ hr. The reaction mixture was left overnight at room temp, diluted with water and warmed. The semi-solid residue was chromatographed in benzene solution over alumina (20 g). Elution with benzene–light petroleum (1:3; 200 ml) gave a colorless gum crystallizing from methanol as flakes (40 mg), m.p. 210–211°, $[\alpha]_D^{28}$ −7·6° (*c*, 0·3). Identity with 3β,28–diacetoxy-16-oxo-oleana-12-ene (IIIb) was established by m.p., mixed m.p. and optical rotation. (Found: C, 75·32; H, 9·75. $C_{34}H_{52}O_5$ requires: C, 75·55; H, 9·63%).

Further elution of the column with chloroform–benzene (1:1; 50 ml) and crystallization of the residue from methanol yielded needles (15 mg) of (IIIc), m.p. 219–220°. (Found: C, 77·43; H, 10·28. $C_{32}H_{50}O_4$ requires: C, 77·11; H, 10·04%).