Tetrahedron Letters No.23, pp. 1829-1836, 1965. Pergamon Press Ltd.
Printed in Great Britain.

THE STRUCTURE OF ATRACTYLIGENIN.

F.Piozzi and A.Quilico
(Istituto di Chimica, Politecnico di Milano)
T.Ajello and V.Sprio
(Istituto di Chimica Farmaceutica, Università di Palermo)
A.Melera
(Varian AG, Zürich)

(Received 6 April 1965)

Preliminary communications (1) have been published by us on the constitution of atractyligenin $C_{19}H_{28}O_4$, the aglycone of atractyloside; more recently a note (2) covering the results obtained by classical chemical work and the relative experimental part has been published. We wish now to present the later results obtained by both chemical and physico-chemical methods.

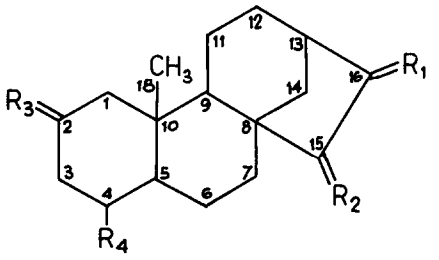
Previous work (1,2) had suggested for this nor-diterpenoidic acid a tetracyclic phyllocladene-like structure, carrying a carboxy group at C_4, an angular methyl group at C_{10}, an hexocyclic methylene at C_{16} and two secondary hydroxy groups in not defined positions. The structure of the carbon skeleton was supported by the obtention of many phenanthrenic hydrocarbons (among them retene) by selenium dehydrogenation.

NMR determinations (3) on several derivatives confirm the structure proposed for atractyligenin (I) and hydroatractyligenin (II); they allow to place the two hydroxy groups at C_2 and C_{15} respectively (4).

Atractyligenin (I), m.p. 189°, has only one C-CH_3 (Kuhn-Roth) and one double bond (hydrogenation, perbenzoic acid oxidation), while hydroatractyligenin (II) $C_{19}H_{30}O_4$, m.p. 237°, has two C-CH_3 and no double bond.

Atractyligenin methylester $C_{20}H_{30}O_4$ (III), m.p. 158°, presents in

its NMR spectrum at 60 Mc a singlet at 0.92 δ (tertiary methyl), a singlet at 3.68 δ ($-COOCH_3$), a slightly broadened signal centered at 3.86 δ ($\geq C_{\underline{x}}H-OH$), a complex multiplet at 4.23 δ ($\geq C_{\underline{y}}H-OH$) and two slightly broadened signals at 5.08 resp. 5.24 δ (two protons of $\geq C=CH_2$).



I	R_1	CH_2	R_2	H,OH	R_3	H,OH	R_4	COOH
II		H,CH_3		H,OH		H,OH		COOH
III		CH_2		H,OH		H,OH		$COOCH_3$
IV		H,CH_3		H,OH		H,OH		$COOCH_3$
V		CH_2		O		O		$COOCH_3$
VI		H,CH_3		O		O		$COOCH_3$
XII		Br,CH_2Br		H,OH		H,OAc		COOH
XV		CH_2		H,OCH_3		H,OCH_3		$COOCH_3$
XVI		O		H,OCH_3		H,OCH_3		$COOCH_3$
XVII		CH_2		H,OH		H,OH		CH_2OH

Hydroatractyligenin methylester $C_{20}H_{32}O_4$ (IV), amorphous solid, has a singlet at 0.90 δ (tertiary methyl), a doublet centered at 1.14 δ (J_{obs} = 7.5 cps: secondary methyl), the complex multiplet at 4.25 δ of $\geq C_{\underline{y}}H-OH$ and a slightly broadened doublet at 3.25 δ (J = 3.5 cps: $\geq C_{\underline{x}}H-OH$).

A comparison of the spectra of (III) and (IV) shows that upon hydro-

genation the $\geq C_{\underline{x}}H\text{-}OH$ proton shifts from 3.86 to 3.25 δ and that its signal changes from a broadened singlet into a doublet with J = 3.5 cps. This observation suggests that the proton undergoing the shift and the double bond must be closely spaced and that there is one proton in a position to the first in (IV). The downfield shift from the usual position (4.6-4.8 δ) of the $\geq C{=}CH_2$ group gives further evidence that its protons are not far from the $\geq C_{\underline{x}}H\text{-}OH$ group.

The complex multiplet of $\geq C_{\underline{y}}H\text{-}OH$ observed in (III) and (IV) at 4.23 resp. 4.25 δ (or 5.10 δ in the corresponding acetylderivatives $C_{\underline{y}}H\text{-}OAc$), when irradiated with a strong RF field at 2.16 δ (5), does simplify into a triplet (J = 10 cps), clearly indicating that the proton originating this signal must be axial and that there is one additional axial proton on each of the two carbon atoms in a position. This observation is of remarkable value because it restricts the possible location of this proton to C_2, C_6 and C_{11}. NMR determinations at 100 Mc clearly resolve this complex multiplet into a triplet of triplets, indicating that the proton in question is additionally coupled to two other protons with a typical axial-equatorial coupling constant J = 4 cps (6). By neglecting the possibility of a long range coupling with a proton in a more remote position of the molecule, one can eliminate C_6 and C_{11} as possible locations for this oxygen function which has thus to be placed in equatorial position at C_2 (7).

Diketo-atractyligenin methylester $C_{20}H_{26}O_4$ (V), m.p. 159°-160°, obtained by Jones' reagent oxidation of (III), has in its UV spectrum λ_{max}^{234} mμ (ε = 7700), indicating an α,β-unsaturated ketone. Its NMR spectrum presents a singlet at 0.98 δ (tertiary methyl) and two well resolved triplets (J = 1 cps) at 5.33 resp. 5.98 δ for the protons of $\geq C{=}CH_2$, according to the strong deshielding effect of the neighbouring ketogroup and the allylic coupling with $C_{13}\underline{H}$ at 2.75 δ. This accounts unequivocally for the location of the second hydroxy group at C_{15} (8).

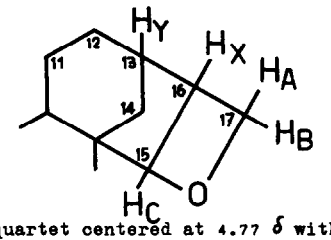
Diketo-hydroatractyligenin methylester $C_{20}H_{28}O_4$ (VI), m.p. 190°, obtained by chromic acid oxidation of (IV) or by catalytic hydrogenation

of (V), has no absorption maximum in the UV spectrum; NMR spectrum: singlet at 0.95 δ (tertiary methyl) and doublet at 1.10 δ (J_{obs} = 7.5 cps: secondary methyl). Upon Huang-Minlon reduction (VI) gives an acid $C_{19}H_{30}O_2$ (VII), m.p. 205°, which by treatment with $LiAlH_4$ affords 19--hydroxy-atractylane $C_{19}H_{32}O$ (VIII), m.p. 99°. Its acetylderivative (IX) shows in the NMR spectrum the singlet of tertiary methyl at 0.95 δ, the doublet of secondary methyl centered at 0.90 δ (J_{obs} = 5 cps) and an eight lines pattern at 3.40-4.20 δ due to the AB part of an ABX system (9) arising from the two oxymethylene protons at C_{19}, coupled with the proton at C_4. The presence of an $-O-CH_2-CH<$ group in (IX) proves that the carboxy group in both (II) and (VII) is secondary. This is confirmed by the $pK^{\ddagger}$ determination (10) giving 7.53 for (VII), in agreement with an equatorial secondary carboxy group (calc. 7.69).

In the 100 Mc spectrum of (III) two additional protons are observed, at 2.71 δ ($C_{13}-\underline{H}$) and 2.66 δ ($C_4-\underline{H}$): the corresponding spectrum of (IV) shows only the C_4 proton at 2.66 δ as a triplet of doublets (J_{ax-eq} = 5.0 cps, J_{eq-eq} = 2.0 cps): this is indicative of an equatorial proton (6, 11), hence the carbomethoxy group is suggested to be axial. On the contrary, this signal is not present in the NMR spectrum of the methylester of (VII). This discrepancy has been solved as we have demonstrated that during the Huang-Minlon reduction the originally axial carbomethoxy group epimerizes into equatorial: under similar conditions (KOH in ethyleneglycol or K ethoxyde in ethanol) both (III) and (IV) give resp. 4-epi-atractyligenin m.p. 240° (X) and 4-epi-hydroatractyligenin m.p. 276° (XI). Hence the carboxy group is axial in (I) and (II), but is equatorial in (VII), (X) and (XI). Accordingly, the C_4 proton resonance in the methylesters of (X) and (XI) is shifted to higher field value at 2.4 δ, while the C_{13} proton is present at 2.75 δ only in the methylester of (X).

Atractyligenin (I) when treated with bromine in acetic acid solution gives dibromo-monoacetyl-atractyligenin $C_{21}H_{30}O_5Br_2$ (XII), m.p. 178°. Treatment of (XII) with Zn and aqueous KOH affords isoatractyligenin

$C_{19}H_{28}O_4$ (XIII), m.p. 232°, a compound containing no double bond and only one hydroxy group, while the fourth oxygen atom is bonded in a cyclic ether bond. Its methylester-monoacetate gives an NMR spectrum which by means of double irradiation (5) can be interpreted in terms of the partial tentative formula:

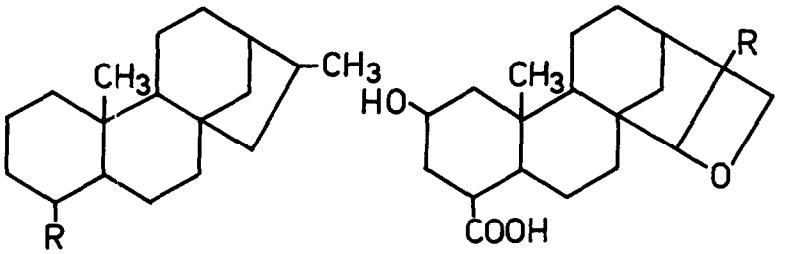


In fact the proton H_A gives a quartet centered at 4.77 δ with J_{AB} = 6.2 cps (12) and J_{AX} = 7.8 cps, the proton H_B a quartet centered at 4.11 δ with J_{AB} = 6.2 cps and J_{BX} = 4.85 cps, and the proton H_C appears as a doublet centered at 4.51 δ with J_{CX} = 4.85 cps. By double irradiation one can show that H_A and H_B are coupled together, as well to an additional proton H_X centered at 2.80 δ. The same experiment, when performed on H_C, also locates the H_X proton at 2.80 δ.

By boiling (XII) with aqueous KOH, a $C_{19}H_{27}O_4Br$ product is obtained, m.p. 218°, to whom structure (XIV) should be attributed; upon treatment with KOH and Zn, (XIV) is converted into isoatractyligenin (XIII). The NMR spectrum of the methylester of (XIV) shows a doublet (J = 1.9 cps) at 4.58 δ ($\geq C_{15}\underline{H}-O-$) and an AB quartet (J = 7.3 cps) at 4.72 resp. 5.02 δ due to the methylene group C_{17}. The absence of further splitting in the resonance of both C_{17} protons places the bromine atom at C_{16}. By double irradiation one can show that the $C_{15}-\underline{H}$ doublet at 4.58 δ simplifies to a singlet when the second RF field is applied at 2.30 δ, a shift attributable to H_Y which is in a position to the newly introduced bromine atom: this splitting could be interpreted as a long range coupling ($^4J \cong 2$ cps), thus suggesting that H_C and H_Y should be <u>cis</u> to each other (13).

The presence of an oxetane ring in (XIII), (XIV) and derivatives is

also supported by two typical strong bands in their IR spectra at 1190 resp. 978 cm^{-1} (14).



VII R = COOH XIII R = H

VIII CH_2OH XIV Br

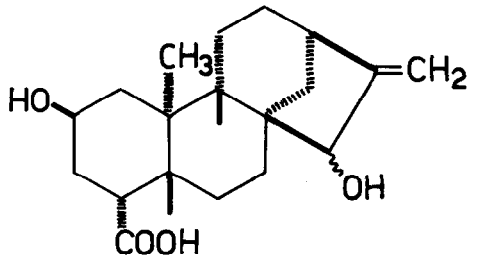
IX CH_2OAc

Chromic acid oxidation of the methylesters of (XIII) and (XIV) gives resp. a monoketone $C_{20}H_{28}O_4$, m.p. 168°, and a monoketone $C_{20}H_{27}O_4Br$, m.p. 207°, which show a strongly negative Cotton effect (resp. $[\alpha]_{309}$ = - 1910° and $[\alpha]_{310}$ = - 1370°); that indicates an antipodal trans-A/B junction with 10α-CH_3 and 5β-H, like in (-)kaurene.

Ozonolysis of the methylester-dimethylether of atractyligenin (XV) $C_{22}H_{34}O_4$ gives the norketone (XVI) $C_{21}H_{32}O_5$ which exibits a positive Cotton effect ($[\alpha]_{350}$ = + 884°) indicating the ββ-$C_{15}C_{16}$ configuration. Atractyligenin possesses hence the usual trans-anti-cis backbone of (-)kaurene and many other tetracyclic diterpenoids.

The configuration of the C_{15} hydroxy group is yet to be completely established. However, the fact that both atractyligenin methylester (III) and atractylitriole $C_{19}H_{30}O_3$ (XVII) (15) do not undergo the allylic rearrangement to the corresponding C_{15}-ketones (16), lets one to consider a 15α-OH (quasi-axial) configuration as very probable. This is also supported by several other results, on which we shall discuss in a forthcoming communication.

Rationalisation of these results would lead to structure (I) for atractyligenin:



Further researches on the stereochemistry of (I) and derivatives are in progress.

We aknowledge financial support of Consiglio Nazionale delle Ricerche (C.N.R.) to our investigations. Our thanks are due to Dr.(Miss) Rosanna Mondelli for some NMR spectra and to Dr.(Mrs.) Luciana Merlini - - Piozzi for helpful collaboration to the chemical work.

References

(1) T.Ajello, F.Piozzi, A.Quilico and V.Sprio, Rend.Accad.Lincei VIII 28, 454 (1960) and La Chimica e l'Industria (Milano) 42, 876 (1960); F.Piozzi, communication at the 1st Symposium on Natural Substances (Sydney, August 1960).

(2) T.Ajello, F.Piozzi, A.Quilico and V.Sprio, Gazz.Chim.Ital. 93, 867 (1963).

(3) The NMR spectra were recorded in $CDCl_3$ solution with TMS as internal standard on Varian Associates A-60 and HR-100 spectrometers. The chemical shifts are given in δ units from TMS and are taken to be positive in the direction of decreasing magnetic field. The double irradiation experiments (5) were carried out on a Varian Associates HR-60 spectrometer by means of a modified Varian V-3521 Integrator.

(4) According to the usual numbering, compare V.P.Arya and H.Erdtman in "Recent Progress in the Chemistry of Natural and Synthetic Colour-

ing Matters and Related Fields", p. 359. Academic Press, New York (1962).

(5) R.Freeman and D.Whiffen, Mol.Phys. 4, 32 (1961).

(6) H.Booth, Tetrahedron 20, 2211 (1964) and references given therein.

(7) For a similar case of an equatorial hydroxy group at C_2, compare NMR of isorosenolic acid, A.I.Scott, D.W.Young, S.A.Hutchinson and N.S.Bhacca, Tetr.Letters 1964, 849.

(8) Compare the UV values given by K.Wiesner, R.Armstrong, M.F.Bartlett and J.A.Edwards, J.Am.Chem.Soc. 76, 6068 (1954), for an identical structure in garryine.

(9) J.A.Pople, W.G.Schneider and H.J.Bernstein, High Resolution NMR p. 132. McGraw Hill, New York (1959).

(10) By the courtesy of Dr. W.Simon, E.T.H. Zürich.

(11) E.L.Eliel and M.H.Gianni, Tetr.Letters 1962, 97.

(12) Compare P.Yates and A.G.Szabo, Tetr.Letters 1965, 485, for a similar oxetane ring with J_{AB}= 6.5 cps.

(13) S.Sternhell, Rev.Pure and Applied Chem. 14, 15 (1964).

(14) G.M.Barrow and S.Searles, J.Am.Chem.Soc. 75, 1175 (1953); P.Yates and A.G.Szabo, Tetr.Letters 1965, 485.

(15) Obtained by $LiAlH_4$ or Na-ethanol reduction of (III).

(16) In the field of the diterpene alkaloids having the same absolute configuration of (-)kaurene, the ones with 15β-OH (garryfoline, songorine, napelline) do easily rearrange to the C_{15}-ketones, while the ones having 15α-OH (garryine and veatchine) do not.