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NEW TRITERPENES OF ALISMA PLANTAGO-AQUATICA L. VAR. ORIENTALE SAMUELS.

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During the course of our studies on the constituents of plant sources related to Chinese drugs, several new triterpenes have been isolated from the rhizomes of Alisma Plantago-aquatica L. var. orientale Samuels. (A. orientale Juzep., Alismataceae). The present communication deals with their structures elucidated on both chemical and X-ray crystallographic techniques.

Repeated silica-gel chromatography of the lipid fraction from the material afforded the five new compounds listed in Table I; the molecular formulae being established by mass spectrometry and elemental analyses.

TABLE I

Compound	Formula	M.p.	$[\alpha]_D$ *1
Alisol A (1)	$C_{30}H_{50}O_5$	amorphous	+99°
Alisol B (2)	$C_{30}H_{48}O_4$	166-168°	+130°
Alisol A monoacetate (3)	$C_{32}H_{52}O_6$	194-196°	+86°
Alisol B monoacetate (4)	$C_{32}H_{50}O_5$	162-163°	+121°
Epi-alisol A (5)	$C_{30}H_{50}O_5$	amorphous	+81°

Alisol A (1) did not crystallize; however, on acetylation the crystalline alisol A triacetate (6), $C_{36}H_{56}O_8$, m.p. 231-233°, $[\alpha]_D$ +56°, was obtained. Evidence for the secondary nature of three acetylable hydroxyls was provided by the NMR spectrum*2 of 6,

*1 The specific rotations were taken on $CHCl_3$ solutions at 21-28°C.

*2 The NMR spectra were measured at 100 mc./sec. in $CDCl_3$ solutions and calibrated against internal tetramethylsilane.

which reveals the doublet at 4.60 (1H) and complex signals (total 2H) near 4.8 p.p.m. (δ). The fourth hydroxyl was considered a tertiary one because the hydroxyl band (3500 cm^{-1}) appeared in the IR spectrum*3 of 6, and no signals assignable to a carbinyl proton was noticed in the NMR spectrum of 6.

The fifth oxygen in 1 was assigned to a six-membered ring ketone by the following evidence: 1 exhibits a carbonyl band at 1705 cm^{-1} in its IR spectrum. Reduction of 6 with $NaBH_4$ followed by acetylation gave the pentol tetraacetate (7), $C_{38}H_{60}O_9$, m.p. 193-194°. Both ORD and CD curves of 6 showed strong positive Cotton effects [RD in dioxane (c=0.217 %), 26°: $[\alpha]_{589}$ +55°; $[\alpha]_{316}$ +805°; $[\alpha]_{308}$ +712° (shoulder); $[\alpha]_{272}$ -643°; $[\alpha]_{255}$ -505°. CD in dioxane (c=0.217%): $[\theta]_{291}$ +8500]. The curves suggest the presence of a 5α-3-ketone moiety like some steroids or triterpenes in the molecule. Further, 1 gave the positive Liebermann-Burchard reaction, and was positive in the Zimmermann test[1].

On oxidation of 1 with HIO_4 in dioxane, acetone and the tetranoraldehyde (8), $C_{26}H_{40}O_3$, m.p. 190°, $[\alpha]_D$ +129°, IR ν_{max}^{KBr} cm^{-1}: 3500 (OH); 1700 (ring ketone); 1720 and 2770 (aldehyde), were obtained. The NMR spectrum of 8 revealed a triplet at 9.58 (1H) and a sextet at 3.80 p.p.m. (1H), the latter being reasonably accounted for the part structure [-CH-C<u>H</u>(OH)-CH_2-].

Treatment of 1 with BF_3-ether complex in acetone followed by acetylation gave the acetonide monoacetate (9), $C_{35}H_{56}O_6$, m.p. 213-215°, $[\alpha]_D$ +75.5°, IR ν_{max}^{Nujol} cm^{-1}: 3480(OH); 1745 (acetyl); 1705 (ring ketone), which showed the NMR signals at 3.34 (1H doublet, <u>H</u>a), and 3.59 p.p.m. (1H multiplet, <u>H</u>b). These signals can well be assigned to the two carbinyl protons involved in the acetonide moiety.

These observations led us to an assumption that alisol A (1) would be a tetracyclic triterpene in which the iso-octyl side chain bears the glycerol moiety. In addition, 1 showed eight C-methyls in the region of 0.95 to 1.22 p.p.m. in its NMR spectrum, while 8 exhibited six methyls.

The aforementioned conclusions receive further support by mass spectrometric studies of 1 and 8, because the compounds showed relatively intense mass peaks at m/e 329 [$C_{22}H_{32}O(OH)^+$] and at m/e 311 ($C_{22}H_{31}O^+$), the latter being accounted for the loss of water from the former.

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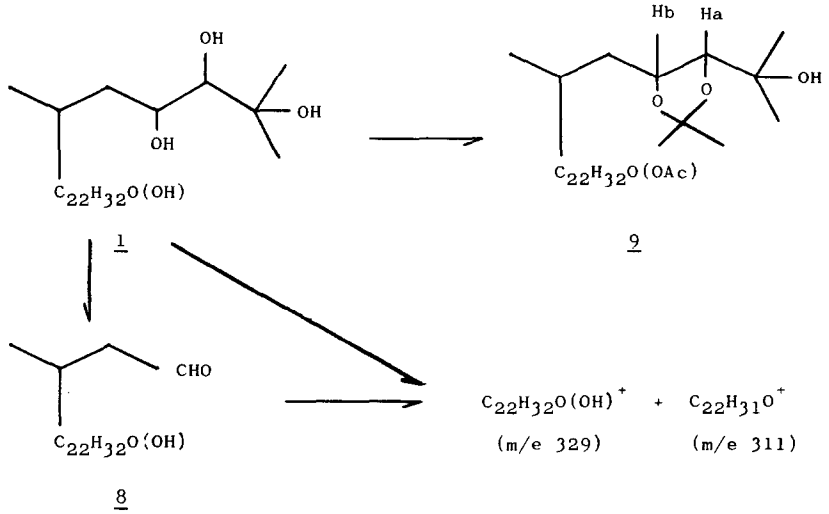
*3 Unless otherwise noted, the IR spectra were taken on $CHCl_3$ solutions.



Both 1 and 6 showed a weak absorption band at 1660 cm^{-1} in their IR spectra; however, no signals assignable to an olefinic proton could be noticed in their NMR spectra. This demonstrates that 1 possesses a tetrasubstituted double bond. Actually, 1 gives the positive Tortelli-Jaffe reaction, though weakly. As to the location of the double bond the position 8(9) can almost certainly be excluded because Δ^8-lanosten-3-one[2] shows the CD curve quite different from that of 6. These observations suggest that a dammarane or a C_{30}-fusidane[3] skeleton is more likely rather than a lanostane, an euphane or a tirucallane carbon skeleton at this stage. This implies that 1 would have the double bond at the 13(17) position. The secondary hydroxyl in the cyclic ring has now to be placed at C_6 or C_{11}. One can deduce this hydroxyl has an equatorial orientation because the carbinyl proton in 8 showed the two large, equal splittings (J=13.5 c.p.s.), occurring along with a third axial-equatorial interaction of 5.6 c.p.s., as is seen in the 11-axial proton resonance in the NMR spectrum of Δ^4-androsten-11α-ol-3,20-dione[4]. However, these discussions did not lead us to any crucial evidence for the ring system of the compound, and the stereochemistry of the side chain also remained unclarified.

The X-ray analysis to establish the structure of alisol A was started at the stage when its acetonide monobromoacetate (10) was found to form well-shaped, stable crystals.

Alisol A acetonide monobromoacetate (10), $C_{35}H_{55}O_6Br$, M=651.71 was crystallized from methanol to afford orthorhombic pillars of m.p. 185-186°. The unit cell containing four

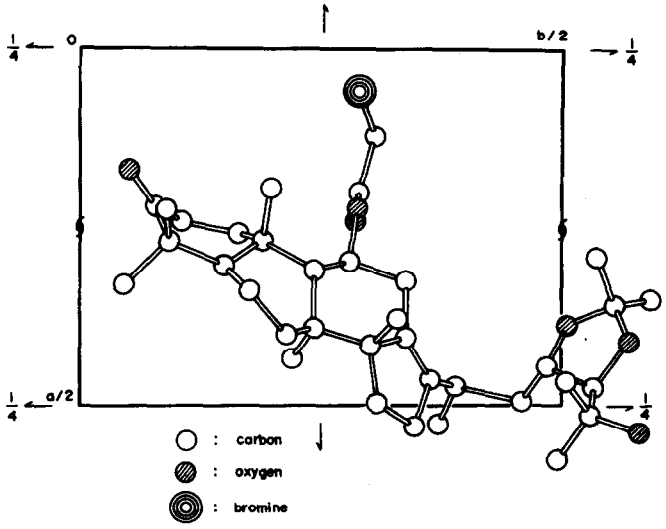
molecules, is of the dimensions, a=18.18, b=24.63 and c=7.75 Å, and the space group is $P_{2_1 2_1 2_1}$. The density measured by the floatation method gave 1.247 (calcd. 1.243) g/cm^3.

The diffractions were measured with Hilger & Watts' linear diffractometer (MoKα-radiation), and 2237 data were collected about the c-axis. For the determination of the coordinates of the bromine atom, a three-dimensional sharpened Patterson function was calculated from these data which had been converted into the absolute scale by the statistical methods.

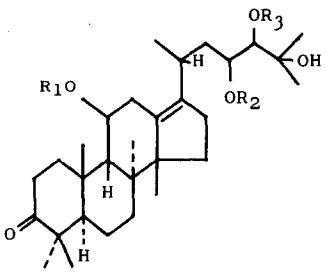
The structure of the molecule was elucidated by the successive applications of minimum function, least squares methods and a Fourier synthesis, the R-value being 0.142 at this stage. The absolute configuration of the molecule was determined by using the anomalous dispersion of the bromine. The stereo-model of 10 is shown in Fig. 1, illustrating also its absolute configuration.

It was hardly possible to establish crystallographycally beyond all doubt the correct assignment of the three atoms at the end of the side chain. However, the chemical evidence that the periodate oxidation of 1 evolved acetone together with the tetranoraldehyde (8) clearly demonstrated that one of the three atoms is oxygen and the other two are carbons. Thus we concluded that the atom having the shortest bond length was oxygen.

FIG. 1



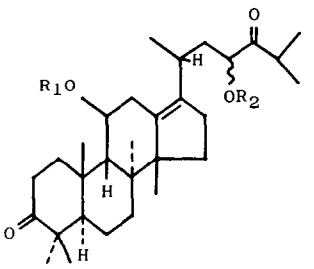
The structure of alisol A was thus established as 1, the absolute configurations of the side chain being 20R, 23S and 24R. Furthermore, it should be noted that both A and B rings of the molecule were in the boat conformation. So far as the authors know, it is the first C_{30}-triterpene having the fusidane-type B/C ring juncture together with a double bond at 13(17).



1: $R_1=R_2=R_3=H$

3: $R_1=R_3=H$, $R_2=Ac$

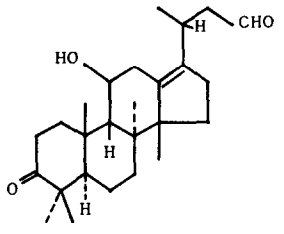
6: $R_1=R_2=R_3=Ac$



2: $R_1=R_2=H$

4: $R_1=H$, $R_2=Ac$

12: $R_1=R_2=Ac$



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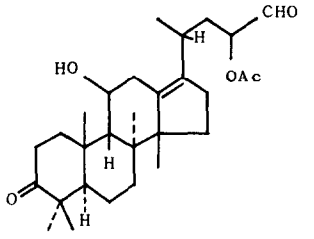

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That the alisol A monoacetate (3) is alisol A 23-acetate, was established by the following evidence: (a) 3 was treated with K_2CO_3 in methanol to afford 1; (b) on acetylation 3 gave the alisol A triacetate (6); (c) oxidation of 3 with HIO_4 yielded acetone and the amorphous trisnoraldehyde (11) which revealed the NMR signals (one proton each) at 9.40 (CHO), 4.61 (CH-OAc) and 3.80 p.p.m. (CH-OH). The last signal is apparently due to the proton at C_{11}, because it is also visible in the NMR spectrum of 3.

The new structural features manifested by alisol A are also present in alisol B (2). On acetylation 2 gives alisol B diacetate (12), $C_{34}H_{52}O_6$, m.p. 140-142°, $[\alpha]_D$ +117.5°,

which shows the CH-OAc protons at 4.85 and 4.49 p.p.m., respectively, in its NMR spectrum. 2 was oxidized with HIO_4 to give the tetranoraldehyde (8), indicating that the tetracyclic ring system of alisol A (1) does also exist in the molecule of 2. Consequently, an α-ketol must be present in the side chain of 2. Thus alisol B is assigned the structure 2.

The alisol B monoacetate (4), on acetylation afforded alisol B diacetate (12), and on treatment with K_2CO_3 gave 2. That 4 is alisol B 23-acetate was concluded by the fact that the NMR spectrum of 4 showed the protons attached to C_{11} and C_{23}, at 3.80 and 4.49 p.p.m., respectively.

Finally, epi-alisol A (5) was acetylated to give epi-alisol A triacetate (13), $C_{36}H_{56}O_8$, m.p. 192-194°, $[\alpha]_D$ +67.5°, the mass spectrum of the latter being superimposable with that of alisol A triacetate (6). Since 5, on oxidation with HIO_4, yielded the tetranoraldehyde (8) and acetone, it was concluded that 5 is an epimer of alisol A at either C_{23} or/and C_{24}.

Further studies of these and related compounds will be reported in the near future.

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