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ON THE CONFIGURATION OF THE 16-HYDROXYL GROUP IN TRITERPENES: A REVISION

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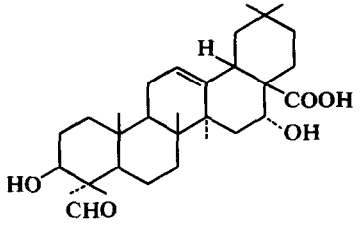
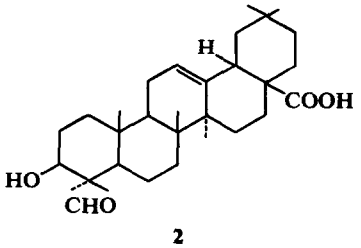
Abstract—Quillaic acid methyl ester was reduced with LAH to Δ^{12}-oleanene-3,16,23,28-tetrol (**4**), which was converted by 2,2-dimethoxypropane to the corresponding diacetonide (**5**). This indicates that the correct orientation of the 16-hydroxyl group in quillaic acid is β-equatorial (**1b**). As a consequence of these results a revision in the stereochemical assignment of the 16-hydroxyl group in a series of triterpenes is suggested.

In a series of papers[1] we have described our studies on the correlation between hemolytic activity and structure of steroids and triterpenes. Our investigation showed that the extent of hemolytic activity is highly influenced by the presence of polar functions in the molecule. We have demonstrated that although the presence of one polar group is necessary for obtaining hemolysis, additional polar functions in the molecule always cause a decrease in the hemolytic property. Thus the activity of bile acids diminishes in the order: 5α-cholanic acid > lithocholic acid > desoxycholic acid > cholic acid. Similar correlations were found for all series of steroids and triterpenes tested.

Contrary to those results, Schlosser and Wulff reported[2] that 16α-hydroxytriterpene-28β-carboxylic acids possess a considerably higher hemolytic potency than the corresponding triterpene carboxylic acids lacking the 16α-hydroxyl group. We have confirmed this observation using quillaic acid [currently accepted formula] (**1a**) and gypsogenin (**2**) as model compounds.[3]



1a



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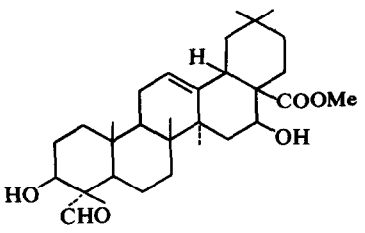
Enhancement of the hemolytic activity of a triterpene 28-carboxylic acid by a 16-hydroxy group can only be rationalized, in accordance with our results, by assuming the formation of an intramolecular hydrogen bond between these two polar functions, thereby reducing the polarity of the compound. Such an intramolecular hydrogen bond cannot be formed between the 16α-hydroxy and 28β-carboxyl groups that are *trans*-diaxial. On the other hand the 28β-carboxyl group could easily be bound by a hydrogen bond to a 16-hydroxyl group, if the latter were β-equatorial. Therefore, we have considered that the stereochemical assignment of the 16-hydroxyl group in triterpenes warrants reinvestigation.

Because of the availability of *Quillaja Saponaria* mol., we have selected quillaic acid as a model compound for our experiments.

The isolation of quillaic acid was carried out following the procedure of Elliot and Kon.[4] Esterification by MeI and K_2CO_3 in MeOH afforded methyl ester **3**, which was reduced by LAH, in ether, to tetrol **4**. This tetrol was converted to the diacetonide derivative **5**, in 70% yield, by treatment with 2,2-dimethoxy-propane in acetone in the presence of a catalytic amount of *p*-TsOH.

The NMR spectrum of **5** showed the typical vinylic proton of C-12 at δ 5·36 ppm, thus confirming that no acid-catalyzed isomerization to the $\Delta^{13(18)}$ compound had taken place.[5] Furthermore, the NMR spectrum exhibited the required signals for 6 protons α to oxygen, in the region of δ 4·1–3·2 ppm, and the isopropylidene methyl groups at δ 1·40 and δ 1·48 ppm, in addition to the signals common for triterpenes.

The mass spectrum showed the parent peak at $m/e = 554$, thus confirming the molecular weight of diacetonide **5**. In addition, peaks were observed at $m/e = 539$ (M—CH_3); $m/e = 496$ (M—acetone); $m/e = 481$ [M—(CH_3 + acetone)], and $m/e = 290$



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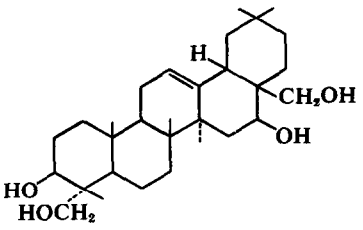

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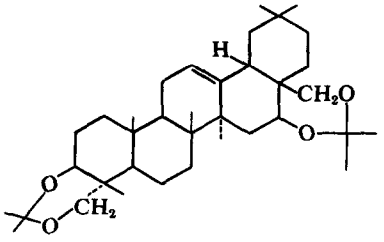

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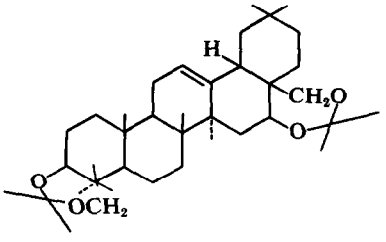
resulting from retro Diels-Alder type fragmentation of the molecule,[6] thus corroborating the position of the double bond.

Hydrolysis of the diacetonide 5 in EtOH—AcOH—H_2O gave tetrol 4 as sole product, thus providing chemical evidence that no rearrangement occurred during the acetonide formation.

The facile formation of diacetonide 5 from tetrol 4, is not compatible with the currently accepted structure of quillaic acid and of related compounds.

It has been established unequivocally,[7] that the D/E ring junction in these compounds (β-amyrin derivatives) is *cis*, and that the angular substituents at C-17 and C-18 are β-oriented. Consequently, the C-28-carboxylic acid and the 16α-substituent are *trans* diaxially related.



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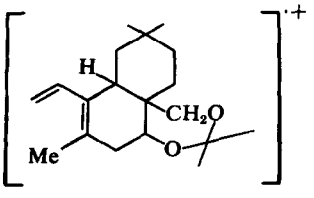


m/e = 290

The configuration of the 16-hydroxyl group in a series of triterpenes, namely: albigenic acid (6),[8] cyclamiretin (7),[9] primulagenin (8)[10,11] and quillaic acid (1b)[11,12] has been found to be identical with that of echinocystic acid (9) and thus was previously assigned 16α-orientation. On the other hand, cochalic acid (10),[13] gummosogenin (11),[14] longispinogenin (12),[14] maniladiol (13),[14,15] chichipegenin (14)[16] and myrtillogenic acid (15)[17] were found to be epimeric with echinocystic acid (10) at C-16 and were therefore assigned a 16β-configuration.

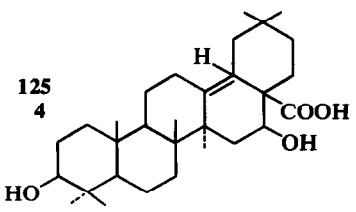
Reduction of the carboxyl group of echinocystic acid (10) to a methyl group lead to an epimer of maniladiol (13). Since maniladiol was found to be acetylated with extreme ease to give the 3,16-diacetate, (while the difference in reactivity of the C-3 and C-16 hydroxyl groups in echinocystic acid was found to be much more pronounced), it was suggested that the C-16 hydroxyl group in maniladiol (13) is equatorial (β-configuration), and that in echinocystic acid is polar (α-configuration).[15]

Since it is inconceivable that two *trans* diaxial hydroxyl groups may form a cyclic acetonide, our results indicate that the correct orientation of the 16-hydroxyl group in quillaic acid (1) is β-equatorial. This requires the reassignment of the configuration of the 16-hydroxyl group in all the above mentioned compounds. Thus for the following compounds the correct stereochemistry is 16-equatorial: quillaic acid (1b), albigenic acid (6), cyclamiretin (7), primulagenin (8), and echinocystic acid (9). While the correct configuration of the 16-hydroxyl group in the following compounds should be α-axial: cochalic acid (10), gummosogenin (11), longispinogenin (12), maniladiol (13), chichipegenin (14) and myrtillogenic acid (15).

These results also enable us to rationalize the hitherto unexplained observations that several 16,28-dihydroxy triterpenes, previously assigned a 16β-configuration, failed to give acetonides.[16,17]

EXPERIMENTAL

All m.p's were determined on a Thomas Hoover Capillary apparatus, and are uncorrected. NMR spectra, unless otherwise stated, were obtained in $CDCl_3$ on Jeol, JNM-C-60H spectrometer. Chemical shifts are given in ppm downfield from TMS as internal standard. Mass spectra were taken on Varian MAT CH-5 at 70 ev using a direct



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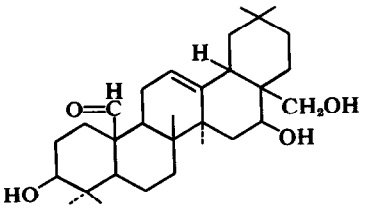

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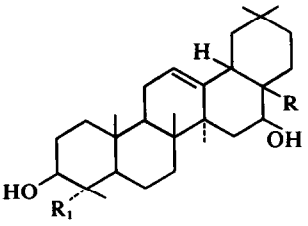
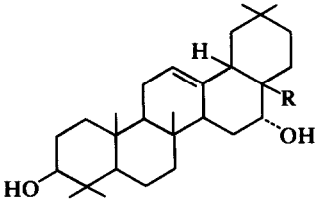
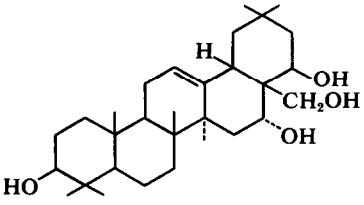


1b, R=COOH; R_1=CHO
8, R=CH_2OH; R_1=Me
9. R=COOH; R_1=Me



10, R=COOH
11, R=CHO
12, R=CH_2OH
13, R=Me



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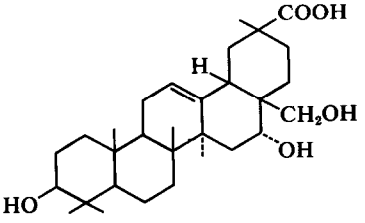

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inlet system. TLC plates were coated with silica gel G (Merck) and the compounds spotted with chlorosulfonic acid. For preparative scale TLC, Merck silica gel GPF_{254} was used.

Quillaic acid methyl ester (3). Crude quillaic acid, dry K_2CO_3 and MeI were stirred, in MeOH, at room temp overnight. After solvent evaporation, the residue was taken up in ether and the ether extract water washed, dried ($MgSO_4$) and evaporated to dryness. Crude solid product was obtained on addition of CCl_4 and was used for the next experiment without further purification. For analysis, a sample was separated by prep TLC using 10% acetone in benzene as the developing solvent, and was crystallized (MeOH) to give the methyl ester derivative m.p. 220–225° (lit.[4] 225°). NMR δ 9·5s 1H (aldehydic), δ 5·4 broad 1H (C-12), δ 4·54 broad and δ 3·8 broad 1H each (α to oxygen C-3 and C-16 unassigned), δ 3·61 s 3H (O—CH_3), in addition to the typical absorptions of triterpenes. m/e = 500 (M^+).

Δ^{12}-Oleanene-3β,16β,23,28, *tetrol* (4). Quillaic acid methyl ester (3) (560 mg) was reduced by LAH (120 mg) in ether (30 ml). After stirring overnight at room temp, the mixture was decomposed and worked up in the usual manner. The solid product (400 mg) obtained after ether evaporation was crystallized from $CHCl_3$-petroleum ether affording the pure tetrol (4) (320 mg), m.p. 254–258° (lit:[5] 246–255°). NMR (acetone-d_6) showed the C-12 vinylic proton at δ 5·26 broad 1H, and 6H α to oxygen between δ 4·2 and δ 3·3 in addition to the usual absorptions of triterpenes.

Acetonidation of 5. To a solution of the tetrol 4 (150 mg) in dry acetone (10 ml), 2,2-dimethoxypropane (1·4 ml) and a catalytic amount of *p*-TsOH were added. A yellow color developed after one min. The reaction was monitored by TLC (5% acetone in benzene). After thirty min at room temp, powdered $NaHCO_3$ was added to neutralize the *p*-TsOH. The mixture was filtered and evaporated, the residue dissolved in $CHCl_3$ and separated by prep TLC using 2% acetone in benzene as the developing solvent. The least polar compound was isolated (120 mg) and crystallized from acetone to give the diacetonide (107 mg), m.p. 205–207° $[\alpha]_D$ +21° (in pyridine). For NMR and mass spectral data see discussion. (Found: C, 77·81; H, 10·60. $C_{36}H_{58}O_4$ requires: C, 77·93; H, 10 54%).

Hydrolysis of the diacetonide 5. To a solution of the diacetonide 5 (20 mg) in EtOH (3 ml), aqueous AcOH (2 ml, 80%) was added. The solution was heated on a steam bath for fifteen min. TLC examination, using benzene: acetone 5:2 showed the presence of one product only, having the same R_f value as that of tetrol 4. This product was isolated by solvent evaporation and crystallized from $CHCl_3$ petroleum-ether to give 13 mg of 4, m.p. 254–256°. A mixed melting point with an authentic sample of 4 showed no depression.

REFERENCES

[1a]R. Segal, M. Mansour and D. V. Zaitschek, *Biochem. Pharmacology* **15**, 1411 (1966); [b]R. Segal, I. Milo-Goldzweig, H. Shupper and D. V. Zaitschek, *Ibid.* **19**, 2501 (1970); [c]R. Segal and I. Milo-Goldzweig, *Ibid.* **20**, 2163 (1971); [d]R. Segal and I. Milo-Goldzweig, *Life Sciences* **10**, Part II, 685 (1971); [e]R. Segal, I. Milo-Goldzweig and M. Seiffe, *Life Sciences* **11**, Part II, 61 (1972).

[2]E. Schlosser und G. Wulff, *Z. Naturforschg.* **24b**, 1284 (1969).

[3]R. Segal, I. Milo-Goldzweig and M. Seiffe, in prep.

[4]F. Elliot and G. A. R. Kon, *J. Chem. Soc.* 1130 (1939).

[5]T. Kubota, H. Kitatani and H. Hinoh, *Tetrahedron Letters* 771 (1969).

[6]H. Budzikiewiez, C. Djerassi and D. H. Williams, *Structure Elucidation of Natural Products by Mass Spectrometry*, Holden Day INc., San Francisco, Vol. 2, p. 122 (1964).

[7]D. H. R. Barton and N. J. Holness, *J. Chem. Soc.* 78 (1952).
[8]A. K. Barua and S. P. Raman, *Tetrahedron* **7**, 19 (1959).
[9]D. H. R. Barton, A. Hammond and J. F. McGhie, *J. Chem. Soc.* 5176 (1962).
[10a]R. Tschesche and F. Ziegler, *Ann.* **674**, 135 (1964); [b]M. Margot and T. Reichstein *Pharm. Acta. Helv.* **17**, 113 (1942); [c]B. Bischof and O. Jeger *Helv. Chim Acta.* **31**, 1760 (1948).
[11a]B. Bischof, O. Jeger and L. Ruzicka, *Ibid.* **32**, 1911 (1949); [b]D. F. Elliot, G. A. R. Kon, and H. R. Soper, *J. Chem. Soc.* 612 (1940); [c]P. Bilham and G. A. R. Kon, *J. Chem. Soc.* 1469 (1940), 552 (1941).
[12a]F. P. Coelho, *Rev. Faculdade Cienc. Univ. Coimbra* **18**, 71 (1949); [b]L. Ruzicka, B. Bischof, E. C. Taylor, A. Meyer and O. Jeger, *Coll. Czech. Chem. Comm.* **15**, 893 (1950).
[13]C. Djerassi, G. H. Thomas and H. Monsimer, *J. Am. Chem. Soc.* **77**, 3579 (1955).
[14a]C. Djerassi, L. E. Geller and A. J. Lemin, *Chem. and Ind.* 161 (1954); [b]C. Djerassi, L. E. Geller and A. J. Lemin, *J. Am. Chem. Soc.* **76**, 4089 (1954).
[15]C. Djerassi, R. M. McDonald and A. J. Lemin, *Ibid.* **75**, 5940 (1953).
[16]A. Sandoval, A. Manjarrez, P. R. Leeming, G. H. Thomas and C. Djerassi, *Ibid.* **79**, 4468 (1957).
[17]C. Djerassi and H. G. Monsimer, *Ibid.* **79**, 2901 (1957).