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STRUCTURE OF SPERGULAGENIN A ISOLATED FROM MOLLUGO SPERGULA L. POSSESSING A NEW MIGRATED HOPANE SKELETON

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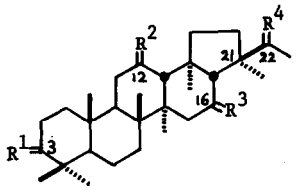
Previously we reported[1] a biogenetic-type transformation of spergulagenic acid to eupteleogenin, a monomethyl ester of the former being isolated as one of the root sapogenols of Mollugo spergula L. In continuing studies of the sapogenol constituents of the plant, we have isolated a new sapogenol which, from its physical properties and those of several derivatives, is considered to be identical with spergulagenin A initially isolated from the same plant source by two Indian groups.[2] In this communication, we wish to report the structure of spergulagenin A(1) possessing a new migrated hopane skeleton on the basis of chemical and X-ray structure evidence.

Spergulagenin A(1), $C_{30}H_{50}O_4$[3], mp. 278-279.5°(from MeOH), $[\alpha]_D$ -15.7°($CHCl_3$), possesses seven tertiary methyls(PMR), three equatorial hydroxyls[2](IR and PMR of triacetate(2)),and one methyl ketone moiety attached to a quaternary carbon(ν: 1694 cm^{-1} in 1; δ : 2.16(3H, s) in 2). Since no unsaturation was observed(tetranitromethane test), the sapogenol has been assumed to be a saturated pentacarbocyclic triterpene possessing a methyl ketone side chain. In addition, since mollugogenols A, B, and C, isolated from the related plant Mollugo hirta Thunb., have been shown to be hopane-type sapogenols[4], spergulagenin A has been assumed to possess a migrated hopane skeleton(1a) or, less likely, a migrated lupane skeleton(1b).

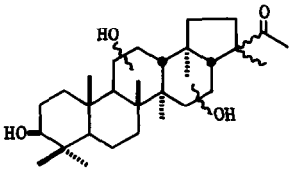
Spergulagenin A(1) was converted to an ethylene ketal(3), which on Ac_2O-pyridine treatment yielded two monoacetates(4,5) and one diacetate(6). One hydroxyl is thus less reactive than

the others, probably due to a bulky ethylene ketal moiety in its vicinity. The mass spectrum of 1 gives a base peak at m/e 413(i, $M^+-CH_3CO-H_2O$) and a prominent ion peak at m/e 207(ii)[5], which, along with biogenetic considerations, a positive Cotton effect of the 3-keto function in 7(prepared from 4 by partial oxidation)($[\Phi]_{303}$ +1592°(peak), $[\Phi]_{265}$ +251°(trough), a= +13.4, c= 0.19 in dioxane)[6] and PMR examination(t-like carbinyl proton at δ 4.45 in 2, 5, 6 and δ 3.15 in 4), shows the presence of 3β-OH in 1. The location of the second hydroxyl has been assumed to be 16β since the coupling pattern of the carbinyl proton in 4(δ 3.75, d.d.d., J= 5.4, 9.6, 10.6 Hz) is similar to that in 16-O-acetyl-6-dehydro-leucotylin(δ 5.20, d.d.d., J= 3.9, 9.2, 12.1 Hz)[7] and another prominent ion peak expressed as iii(m/e 121) is observed in the mass spectrum of 1. Oxidation of 3 with CrO_3-pyridine gave a triketone(8)(ν : 1703 cm^{-1}), which, on Huang-Minlon reduction, gave smoothly 9 having no carbonyl group(IR). Therefore, the third hydroxyl group in 1 has been assumed to be 12β.

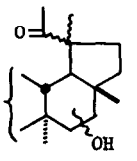
Consequently, the provisional structure of spergulagenin A has been depicted as 1(stereochemistry at C-21 unknown) or less likely as its migrated lupane equivalent. In order to prove the assumption, the X-ray analysis of 4 has been undertaken as described below.



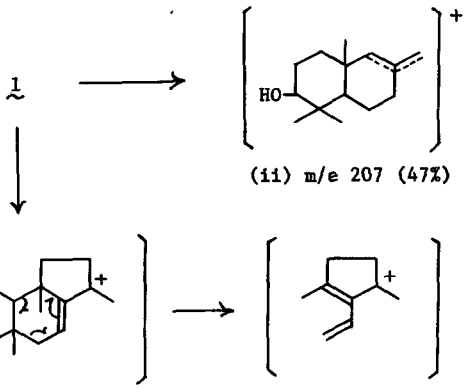
	R^1	R^2	R^3	R^4
1	OH, H	OH, H	OH, H	O
	= spergulagenin A			
2	OAc, H	OAc, H	OAc, H	O
3	OH, H	OH, H	OH, H	O,O
4	OH, H	OAc, H	OH, H	O,O
5	OAc, H	OH, H	OH, H	O,O
6	OAc, H	OAc, H	OH, H	O,O
7	O	OAc, H	OH, H	O,O
8	O	O	O	O,O
9	H_2	H_2	H_2	O,O



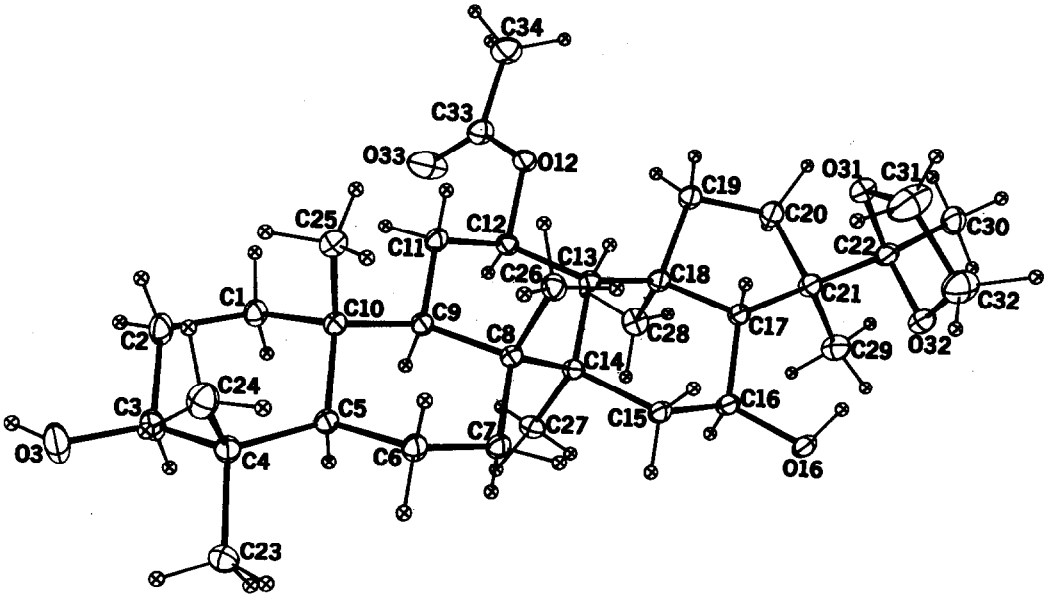
1a



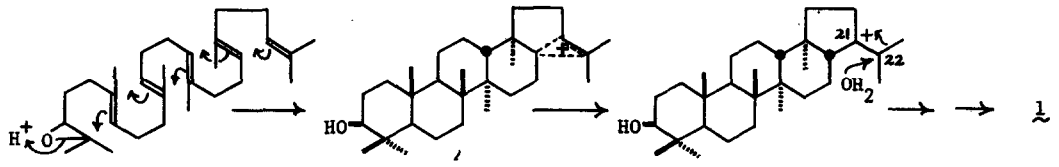
1b



Crystals of 4 are orthorhombic, space group $P2_12_12_1$ with 4 molecules in a unit cell of dimensions a= 9.903, b= 15.127, c= 21.184(Å). 3505 independent structure factors (3201 observed) were collected with an Enraf-Nonius CAD-4 diffractometer using CuKα X-radiation. The structure was solved by direct methods. Initially, unsuccessful attempts were made to solve the structure using two-dimensional cosine invariants computed by the MDKS formula[8]. 64 three-dimensional phases in addition to 48 two-dimensional phases were then determined using the MDKS formula. These phases were used as input to a tangent formula program which gave 187 additional phases. The E-map of the solution with the highest figure of merit showed the triterpenoid skeleton, although not the side chains. Structure factor and Fourier calculations followed by least squares refinement gave the rest of the molecule. All hydrogen atoms were eventually located. The R-value at the conclusion of refinement is 3.7%. The molecular structure and stereochemistry are shown in the figure. A detailed account of the X-ray work will be published later.



Spergulagenin A(1) is the first naturally occurring example of a migrated hopane derivative in which 22-CH_3 is shifted to 21. One can be confident that 1 is a genuine sapogenol since it has been isolated by soil bacterial hydrolysis[9] of the parent saponin(to be reported elsewhere). As for the biogenesis of the new carbon framework, the pathway shown below(iv → v → vi → 1) seems to be attractive.



(iv) (v) (vi)

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