APPENDICES

Appendix 1

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THREE NEW FRIEDELANE LACTONES FROM THE BARK OF GYNOCARDIA ODORATA (FLACOURTIACEAE)

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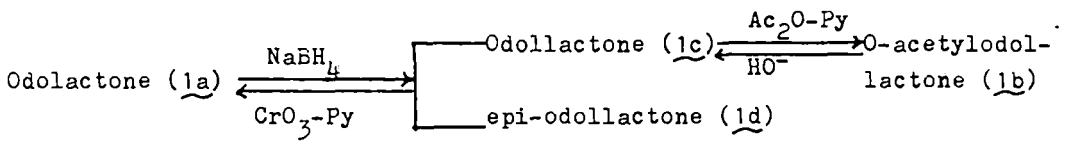
Three new triterpenes - odolactone, acetylodollactone and odollactone from the bark of Gynocardia odorata have been characterised as 3-keto, 3α-O-acetyl and 3α-hydroxyl derivatives of friedelan-26⟶12β-lactone respectively.

The benzene extract of the bark of Gynocardia odorata R. Br.[1] on chromatographic separation yielded odolactone (1a), O-acetylodollactone (1b) and odollactone (1c). Elemental analysis and mass spectrum of 1a showed the molecular formula $C_{30}H_{46}O_3$ (M^+ 454), m.p. 304-05°#, $[\alpha]_D^{CHCl_3}$ -47.06°, IR showed the presence of a saturated six membered ring ketone and a γ-lactone at 1720 and 1760 cm^{-1} respectively. 1b had the molecular formula $C_{32}H_{50}O_4$, m.p. 302-03°, $[\alpha]_D^{CHCl_3}$ -19°, the IR bands at 1760 cm^{-1} showed the presence of γ-lactone and 1725 and 1240 cm^{-1} indicated an acetyl group; 1c was analysed for $C_{30}H_{48}O_3$, m.p. 303-04°, its IR bands at 3490 and 1750 cm^{-1} indicated the presence of a hydroxyl group and a γ-lactone ring respectively. They were inter-related as shown in the Table below:

Table



All the three compounds gave negative TNM test showing the absence of a double bond. ^1H NMR of 1a showed the presence of a secondary methyl at δ0.87 as a doublet (J = 6 Hz), six tertiary methyls at δ0.73, 0.88, 0.96, 0.98, 1.02 and 1.16 as singlets, a proton centered at δ2.4 and two protons at δ2.26 as multiplets. These observations suggested that the compound 1a possesses the

friedelane skeleton. The keto group at the C-3 position was confirmed by the appearance of a methyl at δ 6.8 in the ^{13}C NMR which was due to the methyl at the C-4 position[2] and the large negative Cotton effect ($\Delta\epsilon$ -2.24) in the CD of 1a at 289 nm.

A comparison of the chemical shifts of methyl groups of 1a and that of friedelane derivatives[3] suggested that the C-26 carbon is present as the lactone carbonyl. In the ^1H NMR of 1a (and also of 1b and 1c) there was a triplet centered at δ 4.38 (J = 3 Hz) corresponding to a single proton attached to the carbon bearing the lactonic oxygen. The low coupling constant showed that the proton is equatorial with one axial and one equatorial neighbouring protons, the lactonic oxygen being axial. Considering C-26 as the lactone carbonyl, such a situation is found if the lactonic oxygen is attached either at C-12 or C-16 to form a γ-lactone. The high resolution ^1H NMR (XL-300) of all the three compounds showed two protons at δ 1.76 and 1.85 which couple with each other with a geminal coupling constant (J = 13.4 Hz) and couple with small couplings (J = 2-3 Hz) to an equatorial proton on the carbon with the lactonic oxygen. The peaks of these protons were slightly broadened due to coupling with a nearby axial methyl. These observations suggested that the γ-lactone is formed with C-12, the α-methylene protons on C-11 having such an axial methyl at C-9, whereas the protons on C-15 do not. Thus structure 1a was proposed for odolactone.

The multiplet at δ 3.3 exihibiting large couplings ($W_{\frac{1}{2}}$ = 18 Hz) showed that odollactone possessed an equatorially oriented hydroxyl group at C-3. This was proved by $NaBH_4$ reduction of 1a which afforded two hydroxyl lactones. One that was more polar was identical with odollactone (1c). Acetylation of 1c with Py-Ac_2O furnished an acetate identical with acetylodollactone (1b).

That the lactone carbonyl group is the C-26 has unequivocally been proved by converting (i) odollactone to trichadenic acid[4] (2a), (ii) acetylodollactone to 2a and deoxytrichadenic acid[4] (2b) and (iii) and odolactone to trichadenic acid B[4] (2c) with the help of ethylene diamine-Li under nitrogen atmosphere[5]. The reagent - lithium dissolved in ethylene diamine has been used for the first time to open a lactone ring giving a saturated acid. All the three lactones resisted ring opening both with acids and alkali in alcoholic solutions suggesting that the lactone ring is sterically hindered by the methyl group axially oriented at C-9 and the three hydrogen atoms at C-7, C-16 and C-18 positions which are on the same side as the lactone ring. The easy formation of carboxylic acids (100%) when reduced with Li-$(CH_2NH_2)_2$ confirmed such an environment.

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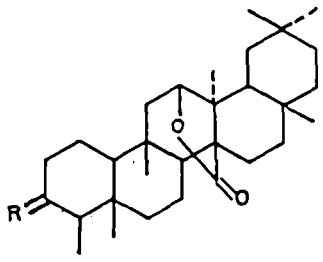
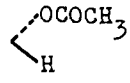
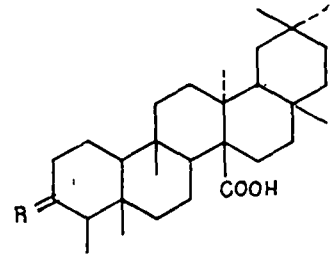


1a, R = O

1b, R = 

1c, R = 

1d, R = 

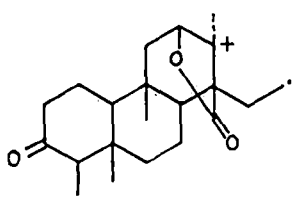


2a, R = 

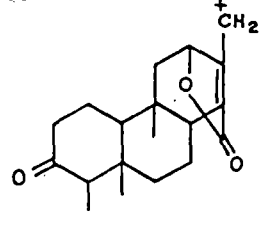
2b, R = H_2

2c, R = 

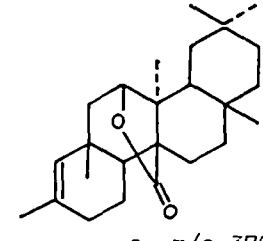
The APT[6] of odolactone showed the presence of 2 $-\overset{|}{C}=O$, 1 $-\overset{H}{\overset{|}{C}}-O$, 6 $-\overset{|}{C}-$, 4 $-\overset{H}{\overset{|}{C}}-$, 10 $-\overset{|}{C}H_2$ and 7 $-CH_3$ in conformity with the structure 1a. The existence of the fragments a and b in the mass spectrum of 1a and the common fragments c, d, e and f[7] in the spectra of 1a, 1b and 1c further supported the proposed structure.



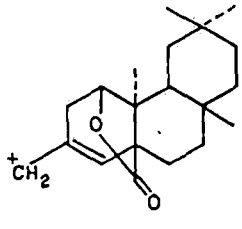
a, m/e 330 (5%)



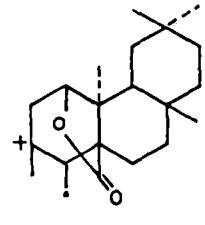
b, m/e 301 (15%)



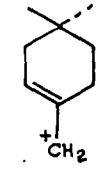
c, m/e 370 (70%)



d, m/e 301



e, m/e 316 (30%)



f, m/e 123 (100%)

It may be mentioned that this is the first report on the isolation of lactones belonging to the friedelane skeleton. That these lactones are not the artifacts of their corresponding hydroxy acids has conclusively been proved by isolating odolactone directly from the benzene extract without treatment with acid/alkali solution : on removal of the solvent under reduced pressure the residue was extracted with ether and the ether insoluble portion on repeated crystallisations from $CHCl_3$-MeOH furnished pure odolactone.

We thank Prof. P. M. Scopes, Westfield College, London for the CD and the optical rotations; Dr. S. K. Sengupta, EIPW Calcutta also for the optical rotations and the Director, CDRI, Lucknow for the mass spectra. One of us (A.H.) is grateful to CSIR, New Delhi for the grant of a fellowship.

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5. Reduction was carried out by heating a mixture of the triterpenoid lactone (100 mg), lithium (100 mg) and dry ethylene diamine (30 ml) under reflux in an atmosphere of nitrogen for 2 hr. The reaction mixture cooled, treated with solid ammonium chloride and then acidified with dil. HCl. The mixture on extraction with ether furnished pure triterpenoid acid.

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   (b) J. N. Shoolery and S. Patt, J. Magn. Reson., 535, 1982.

7. (a) J. S. Shanon, J. G. Macdonald and J. L. Courtney, Tetrahedron Letters, 173, 1963.
   (b) H. Budzinkiewicz, J. M. Wilson and C. Djerassi, J. Am. Chem. Soc., 85, 3688, 1963.

M.ps were recorded in metal block.

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Appendix 2

COMMUNICATED TO TETRAHEDRON LETTERS (1989)

REVISED STRUCTURE OF ODOLACTONE AND SOME NATURALLY OCCURRING TRITERPENOIDS OF THE FRIEDELANE GROUP BASED ON THE APPLICATION OF THE CARBON-CARBON CONNECTIVITY 2D NMR TECHNIQUE

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Structure of odolactone is revised as 3-oxofriedelan-27,15α-olide from the study of the CCC2D plot. This revision of the structure of odolactone leads to the revision of the structures of some other naturally occurring triterpenoids of the friedelane groups; viz., O-acetylodollactone, odollactone, trichadenic acid A, O-acetyltrichadenic acid A, O-acetyltrichadenic acid B, trichadonic acid, trichadenal, O-acetyltrichadenal, 3-oxofriedelan-21α,26-diol, trichadenic acid B, kokoonol, kokoondiol, kokoononol, kokzeylanol and kokzeylanonol.

The structure 1 for odolactone[1], a naturally occurring triterpenoid keto lactone isolated from Gynocardia odorata, was assigned from its spectral analysis and by converting it to trichadenic acid A which is believed to have the structure 3 as reported by Sultanbawa et al[2]. Doubt about the validity of structure 1 for odolactone remained, because in the structure elucidation of trichadenic acids, Sultanbawa et al[2] located the carboxylic carbonyl at C-26 in the friedelane skeleton without any valid scientific ground. However, the fact that odolactone, mp > 320°**, $[\alpha]_D$ - 47.06° ($CHCl_3$), belongs to the class of triterpenoid possessing the friedelane skeleton with the oxo group at C-3 was well documented from a collective view of its spectra[1] — MS, IR, CD and NMR (both ^1H and ^{13}C).

To solve the structural problem of odolactone, the carbon-carbon connectivity two dimensional (CCC2D) NMR technique[3], a powerful structural tool currently offered by NMR, was employed. Figure 1 shows a contour plot of a 75 MHz CCC2D data set for odolactone along with the BBD ^{13}C NMR spectrum at the top. Simple inspection of this plot along with the consideration of general spectral features found in odolactone and in other members of the 3-oxo friedelane group of compounds, unambiguously establishes the structure of odolactone as 2.

The revision of the structure of odolactone as 2 leads to the revision of the structures of its related compounds, isolated from the same source, namely O-acetylodollactone and odollactone as 2a and 2b respectively.

The naturally occurring triterpenoids, namely trichadenic acid A, O-acetyltrichadenic acid A, O-acetyltrichadenic acid B, trichadonic acid, trichadenal, O-acetyltrichadenal, isolated from Trichadenia zeylanica[2], a 3-oxofriedelan-diol from Salacica reticulata[4] trichadenic acid B from Hydnocarpus octandra[5] which are reported to have the structures 3, 3a, 3b, 3c, 3d, 3e, 3f and 3k respectively, are structurally related to odolactone as shown in scheme 1. These structures should be revised to 4, 4a, 4b, 4c, 4d, 4e,

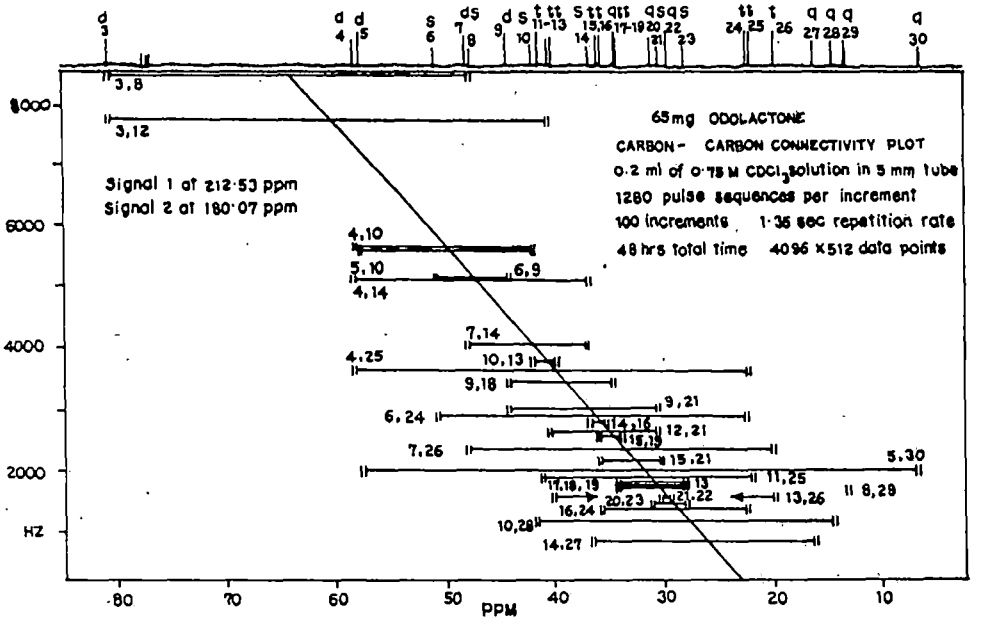


Figure 1. CCC2D SPECTRUM OF ODOLACTONE

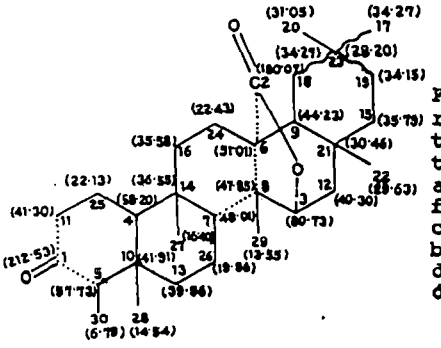
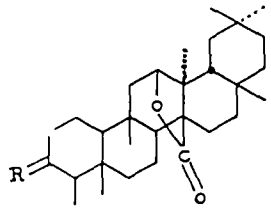


Figure 2. Graphical representation of the result of the CCC2D plot (Fig.1) of odolactone. The numbering of carbons corresponds to a numbering of the signals as they appear in the BBD ^{13}C spectrum from low to high field. Data in parentheses are ^{13}C chemical shifts in ppm relative to TMS. Dotted bonds are missing ones. Wavy bonds are deduced to be represented by superimposed ^{13}C doublets.

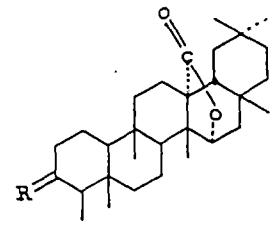
4f and 4k respectively. This study also leads to the conclusion that the structures of kokoonol, kokoondiol, kokoononol, kokzeylanol and kokzeylanonol isolated from Kokoona zeylanica[6,7] should be represented as 3g, 3f, 3h, 3i and 3j respectively, instead of 4g, 4f, 4h, 4i and 4j.



1 R = O

1a R = α-OAC, β-H

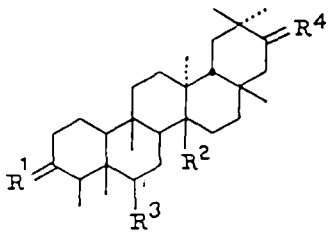
1b R = α-OH, β-H



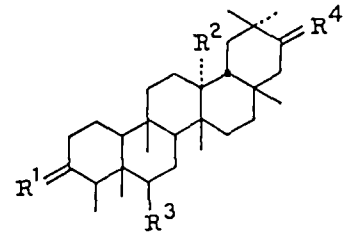
2 R = O

2a R = α-OAC, β-H

2b R = α-OH, β-H

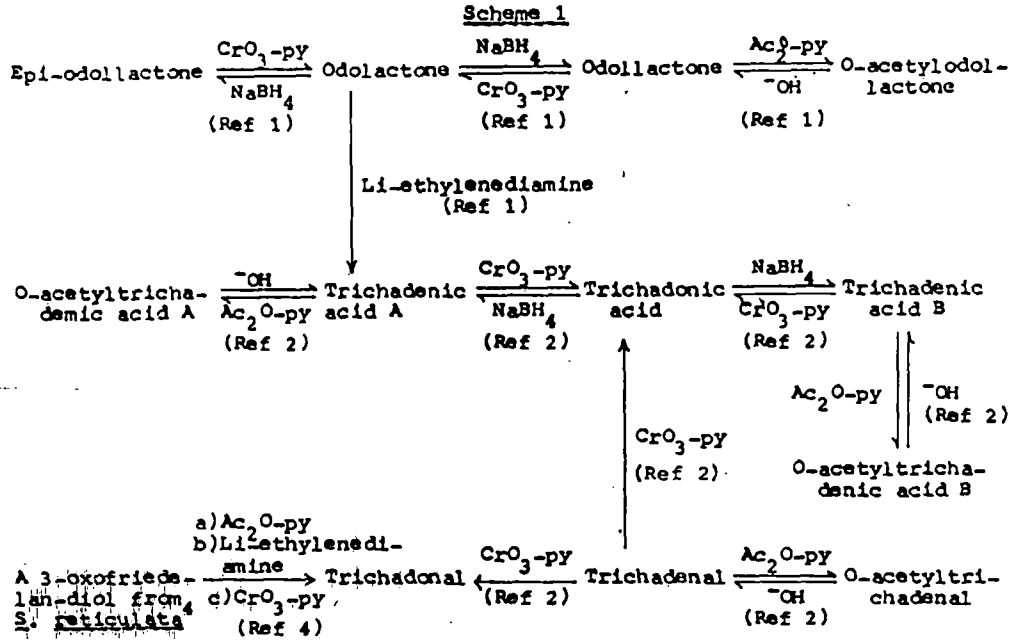


	R^1	R^2	R^3	R^4
3	α-OH, β-H	COOH	H	H_2
3a	α-OAC, β-H	COOH	H	H_2
3b	β-OAC, α-H	COOH	H	H_2
3c	O	COOH	H	H_2
3d	β-OH, α-H	CHO	H	H_2
3e	β-OAC, α-H	CHO	H	H_2
3f	O	CH_2OH	H	α-OH, β-H
3g	O	CH_2OH	H	H_2
3h	O	CH_2OH	H	O
3i	O	CH_2OH	OH	H_2
3j	O	CH_2OH	OH	O
3k	β-OH, α-H	COOH	H	H_2



	R^1	R^2	R^3	R^4
4	α-OH, β-H	COOH	H	H_2
4a	α-OAC, β-H	COOH	H	H_2
4b	β-OAC, α-H	COOH	H	H_2
4c	O	COOH	H	H_2
4d	β-OH, α-H	COOH	H	H_2
4e	β-OAC, α-H	CHO	H	H_2
4f	O	CH_2OH	H	α-OH, β-H
4g	O	CH_2OH	H	H_2
4h	O	CH_2OH	H	O
4i	O	CH_2OH	OH	H_2
4j	O	CH_2OH	OH	O
4k	β-OH, α-H	COOH	H	H_2

Scheme 1



One of us (A.H.) is grateful to the University Grants Commission of India for financial assistance for this work.

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** In the previous communication[1], the melting point of odolactone had been erroneous due to inadvertent oversight. All the lactones from G.odorata have m.p. > 320°. The best identifying characteristics of the lactones are spectral data.

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Appendix 3

REDUCTION OF KETONES TO EPIMERIC ALCOHOLS WITH POTASSIUM HYDROXIDE-DIETHYLENE GLYCOL

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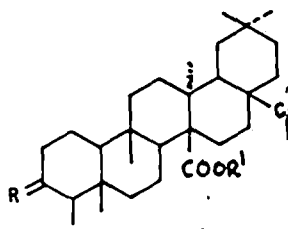
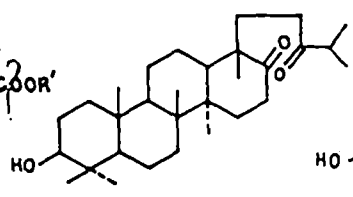
(*Received in UK* 4 *March* 1985)

Abstract - Triterpenoid ketones have been reduced to epimeric alcohols on boiling with potassium hydroxide in diethylene glycol. α,β-unsaturated ketone furnished saturated epimeric alcohols.

During the hydrolysis of 3-keto-methyl trichadenate[1] 1 with potassium hydroxide in diethylene glycol we observed that the 3-keto functional group of the triterpenoid being converted to epimeric alcohols (viz. trichadenic acid A and B[1]) 1a and 1b. A survey of the literature showed that during the degradation of hydroxydiketone 2 with potassium hydroxide in diethylene glycol, Barton et al[2] obtained the dihydroxy compound 3. Similar observation was made by Halsall et al[3] (4 ⟶ 5). Doering et al[4,5] have reported the equillibration of ketones and alcohols in presence of their respective alkoxides under high pressure and temperature.



1, R=O, R'=CH_3
1a, R=α-OH, β-H, R'=H
1b, R=β-H, α-OH, R'=H



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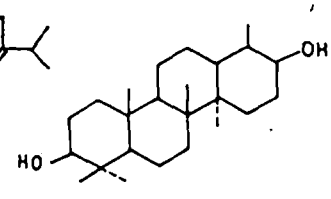

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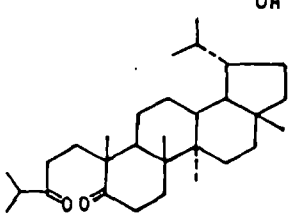

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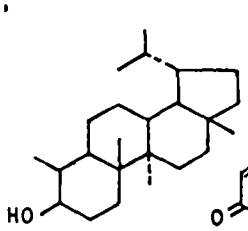

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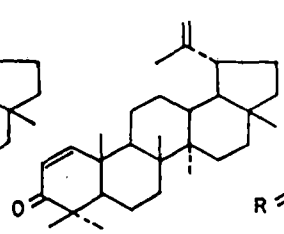

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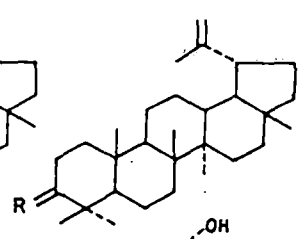


7, R=O 7a, R=α-OH, β-H 7b, R=β-H, α-OH

In order to examine the validity of the reaction on other keto compounds we carried out the reaction on a series of triterpenoid ketones (Entries 1 - 7) and one non-terpenoid ketone (Entry 8) as shown in Table I.

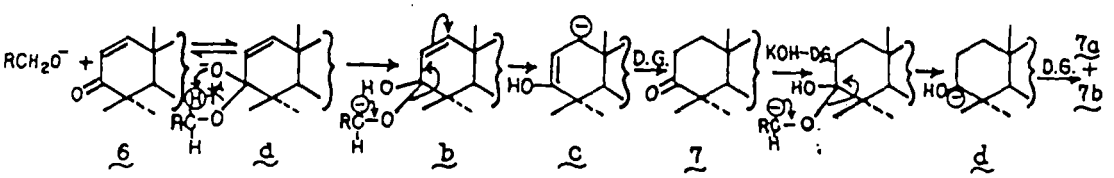
Table I

Entry No.	Ketones	Products (Yield in %)
1	Methyl trichadonate[1] 1	Trichadenic acid A[1] 1a (50) and Trichadenic acid B[1] 1b (15)
2	Friedelin[6]	Friedelanol[6] (45) and epi-friedelanol[6] (20)
3	Moretenone[7]	Moretenol[7] (50) and epi-moretenol[7] (15)
4	Taraxerone[6]	Taraxerol[6] (50) and epi-taraxerol[6] (15)
5	β-amyrone[6]	β-amyrin[6] (50) and epi-β-amyrin[6] (15)
6	Lupenone[6] 7	Lupeol[6] (50) and epi-lupeol[6] (15)
7	Glochidone[8]	Lupeol[6] (40), epi-lupeol[6] (15) and lupenone[6] (8)
8	Benzophenone	Benzhydrol (90)

Thus from the above observation we find that the ketones are reduced to the epimeric alcohols and the formation of the equatorial isomers predominate the less thermodynamically stable axial isomers.

Mechanism:- At the high temperature of the reaction mixture, potassium hydroxide probably forms potassium-glycoxide with diethylene glycol. The glycoxide so formed may reduce the ketone in a cyclic mechanism that may be considered parallel to the action of aluminium-alcoxides in the case of Meerwein-Ponndorf -Verley reduction[9]; but in the latter case the reduction of α,β-unsaturated ketones furnish only the allylic alcohols[10] whereas in the present case the entry 7 shows that along with the keto group, the α,β-unsaturation is also reduced which cannot be explained by the cyclic mechanism. A most probable mechanism for this reduction may follow the route as depicted in the Scheme I :

Scheme I



Under the high thermal condition, the ketone undergoes nucleophilic attack by the anionic glycoxide to form the intermediate anion a that undergoes hydride ion shift forming the carbanion intermediate b ; elimination of glycolaldehyde from b furnishes the tautomeric carbanion c which subsequently acquires a proton from the solvent, the glycol to form the saturated ketone. The isolation of a small amount of the ketone, lupenone 7 from the reaction mixture as shown in entry 7 of Table I confirms the proposed mechanism. Further nucleophilic attack by the glycoxide anion on the saturated ketone in a similar path furnishes the intermediate anion d which ultimately affords the epimeric alcohols.

EXPERIMENTAL

M.ps are uncorrected. IR spectra were run in KBr disc in Beckman-20 and UV spectra were recorded in ethanol in Beckman DU-2 Spectrophotometers. Mass spectra were run in JMS-300 instrument. All the rotations were determined in $CHCl_3$ soln. Column chromatography were performed in silica gel (BDH 60-120 mesh) and TLC were run on plates coated with silica gel G and were developed in iodine chamber. All the analytical samples were routinely dried for 36 h in vacuo. Petrol used had the b. p. 60-80° and the ether extracts were dried over anhydrous Na_2SO_4.

Treatment of methyl trichadonate 1 with KOH-diethylene glycol: Preparation of trichadenic acid A 1a and trichadenic acid B 1b : A mixture of methyl trichadonate (0.5 g) and KOH (2 g) in diethylene glycol (50 ml) was heated in a r.b. flask (100ml) in a heating mantel initially without condenser till all the moisture escaped from the mixture. When the temperature rose above 160° in the vapour phase, the condenser was fitted in the r.b. flask and the mixture was refluxed for 2h. The mixture was then cooled, acidified with dil HCl and extracted with ether. The ether extract was washed with water, dried and the solvent was removed by distillation. The residue (0.45 g) was chromatographed over silica gel column (15 g). The column on elution with benzene:ether (4:1) furnished a solid (0.085 g) which was crystallised from $CHCl_3$-MeOH to afford a solid of m.p. 334-35°, $[\alpha]_D$ +41°, IR: 3425, 1690; M 458. The acetate derivative prepared by Ac_2O-Py method had the m.p. 270-71 $[\alpha]_D$ +50°, M^+ 500; IR: 1725, 1690, 1240 cm^{-1}. The acetate was identified as O-acetyl trichadenic acid B by m.m.p., co-IR and co-TLC with an authentic sample. On further elution of the column with benzene:ether (7:3) a second solid was eluted which after crystallisation from $CHCl_3$-MeOH had m.p. 289-90°, $[\alpha]_D$+ 23°; M^+ 458; IR: 3410, 1688 cm^{-1}. Acetylation of the solid with Ac_2O-Py method and workup in the usual manner furnished an acetate m.p. 251-52°, $[\alpha]_D$ + 27°, M^+ 500; IR: 1740, 1688, 1240 cm^{-1}. The acetate was identified as O-acetyl trichadenic acid A by m.m. p., co-IR and co-TLC with an authentic sample.

Conversion of friedelin to epi-friedelanol and friedelanol: A mixture of friedelin (0.5 g) and KOH (2 g) in diethylene glycol (50 ml) was boiled in a r.b. flask (100 ml) in a heating mantel without condenser till the temperature of the escaping vapour started registering temperature above 160° when the condenser was fitted in. The mixture was allowed to reflux for 2 h and then cooled. The mixture was then acidified with dil HCl, extracted with ether, washed the ether extract with water and dried (Na_2SO_4). The solvent was removed and the residue (0.45 g) was chromatographed over silica gel (15 g). Elution of the chromatogram with benzene:Petrol (1:4) afforded a solid that was crystallised from $CHCl_3$-MeOH to furnish crystals m.p. 278-80°, $[\alpha]_D$ + 20°, IR: 3610 cm^{-1}. The acetate prepared by Ac_2O-Py method had m.p. 291-92°, $[\alpha]_D$ + 38°, IR: 1730, 1240 cm^{-1}; M^+ 470 was found identical (m.m. p., co-IR and co-TLC) with an authentic sample of epi-friedelanol acetate. Further elution of the column with benzene:petrol (3:2) furnished another solid that was crystallised from $CHCl_3$-MeOH had m.p. 300-302°, $[\alpha]_D$ + 18°; IR: 3620 cm^{-1}; its acetate prepared by Ac_2O-Py method had the m.p. 315-16°, $[\alpha]_D$ - 10°; IR: 1730, 1240 cm^{-1} was found identical with an authentic sample of friedelanol acetate by comparison of their m.p., TLC and IR spectra.

Reduction of moretenone to epi-moretenol and moretenol: Moretenone (0.5 g) and KOH (2 g) was mixed with diethylene glycol (50 ml) taken in a r.b. flask(100 ml) and heated as described before. The solid (0.45 g) obtained after usual workup was chromatographed. Elution of the chromatogram with benzene:petrol (1:4) yielded a solid (0.08 g) that was crystallised from MeOH ; the crystals had m.p. 223-24°, $[\alpha]_D$ - 2°, IR: 3460, 3080, 1640,890 cm^{-1}; M^+ 426; its acetate prepared by Ac_2O-Py method had m.p. 231-32°, $[\alpha]_D$- 18°; IR: 3070, 1725, 1640, 890 cm^{-1}; M^+ 468. The acetate was identical (m.m.p., co-IR and co-TLC) with an authentic sample of epi-moretenyl acetate. Further elution of the column with benzene:petrol (2:3) gave a solid (0.25 g) which on crystallisation from $CHCl_3$-MeOH furnished crystals of m.p. 228-30°, $[\alpha]_D$ + 27°; IR: 3480, 3070,1640,890 cm^{-1}; M^+ 426; the acetate prepared by Ac_2O-Py method had m.p. 276-78°, $[\alpha]_D$ + 20°; IR: 3070, 1725, 1640, 1250, 885 cm^{-1}; M^+ 468 was identified as moretenyl acetate by comparison of m.p., IR and TLC with an authentic sample.

Reduction of taraxerone to epi-taraxerol and taraxerol: A mixture of taraxerone (0.5 g) and KOH (2 g) in diethylene glycol (50 ml) was refluxed for 2 h as described above. The solid (0.45 g) obtained after usual workup was absorbed in a chromatogram (15 g) and the column on elution with benzene:petrol (1:4) yielded a solid (0.08 g). The solid on crystallisation from $CHCl_3$-MeOH furnished crystals m.p. 262-64°, $[\alpha]_D$ - 18°, IR: 3470, 810 cm^{-1}; M^+ 426; its acetate prepared by Ac_2O-Py method had m.p. 202-203°, $[\alpha]_D$ - 25°; IR: 1730, 1240, 820 cm^{-1}; M^+ 468 was identical in its m.p., TLC and IR with an authentic sample of epi-taraxeryl acetate. The chromatogram on further elution with benzene:petrol (3:2) yielded a solid (0.25 g) which on crystallisation from $CHCl_3$-MeOH furnished crystals of m.p. 278-79°, $[\alpha]_D$ + 3°, IR: 3460, 815 cm^{-1}. The solid on acetylation with Ac_2O -Py method formed acetate of m.p.297-99°, $[\alpha]_D$ + 8°; IR: 1730, 1240, 820 cm^{-1}; M^+ 468 which was identical (m.m.p., co-IR and co-TLC) with an authentic specimen of taraxeryl acetate.

Conversion of β-amyrone to epi-β-amyrin and β-amyrin: A mixture of β-amyrone (0.5 g) and KOH (2 g) in diethylene glycol (50 ml) was refluxed as described above. The solid (0.45 g) obtained after usual work-up was chromatographed over silica gel (15 g). Elution of the column with benzene:petrol (1:4) gave a solid that was crystallised from MeOH. The crystals had m.p. 220-22°, $[\alpha]_D$ + 70°; IR: 3540, 820 cm^{-1}; M^+ 426; its acetate (Ac_2O-Py) had m.p. 125-26°, $[\alpha]_D$ + 55°; IR: 1730, 1240, 810 cm^{-1}; M^+ 468 was identified (m.m.p., co-IR, and co-TLC) with an authentic sample of epi-β-amyrin acetate). Further elution of the column with benzene:Petrol (2:3) furnished a solid (0.25 g) which was crystallised from $CHCl_3$-MeOH. The crystals had m.p. 197-98°, $[\alpha]_D$ + 80°; IR: 3500, 810 cm^{-1}; Ac_2O-Py

method of acetylation formed an acetate m.p. 237-38°, $[\alpha]_D$+ 79°; IR: 1730, 1240, 820 cm^{-1}; M^+ 468 which was identical (m.m.p., co-IR and co-TLC) with an authentic sample of β-amyrin acetate.

Reduction of lupenone to epi-lupeol and lupeol: A mixture of lupenone (0.5 g) and KOH (2 g) in diethylene glycol (50 ml) was refluxed for 2 h as described above. The product (0.45 g) obtained after usual manner was chromatographed over silica gel (15 g). Elution of the chromatogram with petrol furnished a solid (0.08 g) that was crystallised from MeOH to afford crystals m.p. 200-201°, $[\alpha]_D$ + 17°; IR: 3320, 3060, 1640, 880 cm^{-1}; the acetate prepared with Ac_2O-Py had m.p. 159-60°; $[\alpha]_D$ - 5°; IR: 3060, 1730, 1640, 1250, 880 cm^{-1} was identical (m.m.p., co-IR and co-TLC) with an authentic sample of epi-lupenyl acetate. Further elution with benzene:petrol (2:3) and crystallisation of the solid with $CHCl_3$-MeOH furnished a solid of m.p. 214-16°, $[\alpha]_D$ + 26°; IR: 3340, 1640, 890 cm^{-1} which on acetylation with Ac_2O-Py formed an acetate m.p. 215-17°; IR: 1730, 1640, 1240, 880 cm^{-1} that was identical (m.m.p., co-IR and co-TLC) with lupenyl acetate.

Reduction of glochidone to lupenone, epi-lupeol and lupeol: A mixture of glochidone (0.5 g) and KOH (2 g) in diethylene glycol (50 ml) was refluxed for 2 h as in the previous cases. The product(0.45 g) obtained after usual workup was absorbed in a column of silica gel (15 g). Elution of the chromatogram with petrol furnished a solid (0.04 g) that was crystallised from $CHCl_3$-MeOH to afford fine crystals of m.p. 168-69°; IR: 1700, 1640,880 cm^{-1}; UV: no absorption between 220 - 270 nm; M^+ 424 was identified as lupenone by comparison of m.p., IR and TLC with an authentic specimen. Further elution with petrol furnished solid (0.08 g) that was crystallised from MeOH to give crystals m.p. 199-200°; $[\alpha]_D$ + 18°; IR: 3320, 3060, 1640, 880 cm^{-1}; M^+ 426 was directly compared with an authentic sample of epi-lupeol and was found identical. Elution of the column with benzene:petrol (2:3) yielded a solid (0.20 g)that crystallised from $CHCl_3$-MeOH to afford crystals of m.p. 215-16°, $[\alpha]_D$ + 24°, IR: 3340, 1640, 890 cm^{-1}; the acetate prepared with Ac_2O -Py had m.p. 214-15°: IR 1730, 1640, 1240, 880 cm^{-1} was found identical (m.m.p., co-IR and co-TLC) with an authentic specimen of lupenyl acetate.

Reduction of benzophenone to benzhydrol: A mixture of benzophenone (0.5 g) and KOH (2 g) in diethylene glycol (50 ml) was refluxed for 2 h as in the previous cases. After usual workup the solid (0.45 g) was chromatographed over silica gel (15 g) and elution of the column with benzene:petrol (3:2) furnished solid that was crystallised from $CHCl_3$-MeOH to afford crystals of m.p. 67-68°, IR: 3350, 1605, 1280, 1050,1030, 940, 920, 860, 765, 750, 700 cm^{-1}; M^+ 168. It was found identical (m.m.p., co-IR and co-TLC) with an authentic sample of benzhydrol.

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Appendix 4

Use of Two-Dimensional NMR Spectroscopy for Structure Elucidation of Sepesteonol

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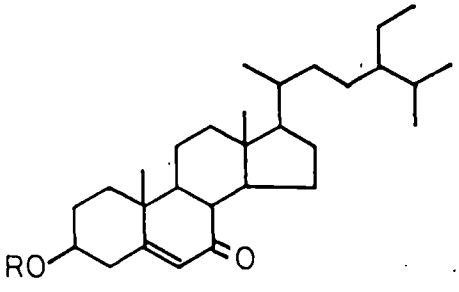
The homonuclear two-dimensional J-spectroscopy in conjunction with attached proton test and ^{13}C NMR data have correctly established the structure of a new sterol, sepesteonol.

The homonuclear two-dimensional J-spectroscopy (HOM2DJ)[1], a recently developed NMR technique results in the separation of proton chemical shifts and spin-spin coupling constants along perpendicular axes. When suitably plotted, the data can often be interpreted unequivocally, in comparison to the normal one-dimensional spectrum wherein it may be necessary to make some assumptions for the interpretation of the data.

We discuss in this communication the use of this technique for the correct evaluation of the structure of a new compound, sepesteonol (I) (isolated from *Flacourtia* ~~*sepiaria*~~ *seperia*[2]), $C_{29}H_{48}O_2$, m.p. 132-33°, $[\alpha]_D$ −98°, λ_{max} 235 nm, ν_{max}^{nujol} 3520, 1655, 1620 cm^{-1}. I on acetylation (Py-Ac_2O) gave sepesteonyl acetate (II), $C_{31}H_{50}O_3$, m.p. 169-70°, $[\alpha]_D$ −92°, ν_{max}^{nujol} 1724, 1655, 1260, 1245 cm^{-1}. PMR spectrum of II exhibited the presence of six methyl groups in the region δ0.66 −1.19, an acetoxy methyl at 2.01, a proton geminal to acetoxyl group at 4.7 as a multiplet and a vinyl proton at 5.7 as a doublet with a small coupling (J = 1.38 Hz). The presence of a C-ethyl moiety in II was confirmed by HOM2DJ experiment. This experiment gave the chemical shifts of the methyls along one axis and spin-spin couplings along the axis at right angle to the first, resulting in a two-dimensional plot, distinguishing the C-26 and C-27 methyl groups as non-equivalent split as two doublets with J = 7.5 Hz by the proton on C-25, while the three peaks at δ0.77, 0.81 and 0.85 were the members of a triplet due to protons at C-29, thereby establishing the presence of a C-ethyl group.



I, R=H
II, R=CH_3CO

The ^{13}C NMR chemical shifts of C-22 to C-29 agreed well with those of a model compound in accordance with Lindeman and Adams's additivity rules[3]. The ^{13}C NMR chemical shifts of the rest of the carbon atoms (C-1 to C-21) of II were very close to those of 3β-acetoxy-cholest-5-en-7-one[4].

The possibility still remained that a 7-acetoxy-4-en-3-one structure might satisfy the spectral data. The 3β-acetoxy-5-en-7-one structure was established by a second two-dimensional experiment, a homonuclear correlation experiment[5] as a contour plot. In this experiment the proton spectrum was displayed along the diagonal of a square plot. The off-diagonal elements represented spin-spin coupling between the protons whose signals on the diagonal had the same x- and y- co-ordinates. This experiment showed a doublet at δ5.7 due to C_6-H, which was spin-spin coupled to the group of signals at 470 Hz (2.5 ppm), and no other protons. The signal at δ2.5 would be expected to arise from the protons on C-4 which would couple to the proton on C-6 with an allylic coupling constant. The proton at δ4.7 (C_3-H) on the other hand showed coupling not only to the protons at C-4 but also to two other protons, viz. axial and equatorial C-2 protons, indicating thereby the presence of four neighbouring protons which could not arise from a 7-acetoxy-4-en-3-one structure.

The C-2 axial and equatorial protons were precisely located by projecting the spots and circles in the contour plot onto either of the axes of the plot which looked like a partial spectrum in which only peaks that coupled to the proton on the diagonal showed up.

The attached proton test (APT)[6] of II showed the presence of 10 methylenes, 9 methines, 7 methyls and 3 tertiary carbons in conformity with the above structure. This is an example where a single instrumental technique has been utilized to elucidate the structure of a compound. The structure (I) for the sterol has further been confirmed by chemical methods which will be reported shortly.

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