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Absolute Structure of Acacic Acid*[1]

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Acacic acid, a pentacyclic triterpenic acid, is present as a saponin in various members of the family Leguminosae, but the highest yield is obtained from the pods of *Acacia concinna* DC. On the basis of degradative studies, transformations, IR, UV, NMR, mass spectrography, and ORD, and CD studies its absolute stereochemistry and structure have been fixed as 3β, 16β, 21β-trihydroxy-18β-olean-12-en-28-oic acid and that in the acetate the rings E and D are in *boat* and *quasi-boat* forms, respectively, with D/E rings *cis* fused.

Acacia concinna DC. commonly known as 'Shikakai' in Hindi is a member of the family Leguminosae, sub-family Mimosacaea. It is a common bush. It grows in tropical jungles, and especially in the southern part of India. The pods are extensively used as a detergent and are preferred to soap by people taking an oil bath.[1] In India the washing of women's hair with the powder of the pods of Acacia concinna is very prevalent as it is said to increase the length of the hair. It is a rich source of saponin which on hydrolysis yields an acid sapogenin (acacic acid).

No work seems to have been done on the saponin and sapogenin contents of this plant except for some works on the standardization of the conditions for commercial isolation of crude saponin.[2] Recently in these laboratories the seeds of this plant were studied and found to contain a new triterpenic acid, acacic acid,[3] which has been isolated from the seeds and bark of Acacia intsia.[3-5] Acacic acid has also been isolated from the seeds of Albizzia spitulata[6] and Albizzia odoratissima.[7] On preliminary studies the pods of Acacia concinna have been found to contain the saponin of acacic acid. Acacic acid was found to be a trihydroxymonocarboxylic acid belonging either to the α-amyrin group or to the tetracyclic triterpene group.[5,8] Later Varshney[9,10] on biogenetic grounds suggested 3,16, 21-trihydroxyolean-12-en-28-oic acid (I) structure for this acid.

Well powdered pods of *Acacia concinna* DC. obtained from Kerala (South India) were extracted exhaustively with ethanol. Removal of the solvent yielded a dark brown coloured semisolid residue, which was treated in the usual manner[10] to yield a fairly pure sample of the saponin. The saponin was then dissolved in a large amount of water and hydrolyzed with sulphuric acid (10%). All attempts to crystallize the saponin failed, and it was subjected to further purification by potassium salt formation. On acidification with hydrochloric acid the alkaline solution of the potassium salt precipitated the acid genin. It was then acetylated with pyridine and acetic anhydride in the cold. The acetate (II) on cyrstallization from methanol gave fine colourless needles mp 235—36°C. It showed a positive tetranitromethane test for a carbon-carbon double bond. It was found to be identical with acacic acid diacetate lactone by mixed m.p. and superimposable infrared spectra.[3,5] The infrared spectrum of diacetate lactone (Fig. 1) showed bands of acetate and γ-lactone at 8.05 μ and 5.6 μ. The

*1 A resume of some of the findings reported here appeared in an advance short communication in *Tetrahedron Let.*, **1965**, 1187—1197.

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1) Wealth of India, C. S. I. R., New Delhi, 1948, Vol. 1, p. 13.

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3) M. O. Farooq, I. P. Varshney and Z. Naim, *Arch. Pharm.*, (Weinheim) **295**, 12, (1962).

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5) M. O. Farooq, I. P. Varshney and Z. Naim, *Arch. Pharm.*, (Weinheim) **294**, 133 (1961).

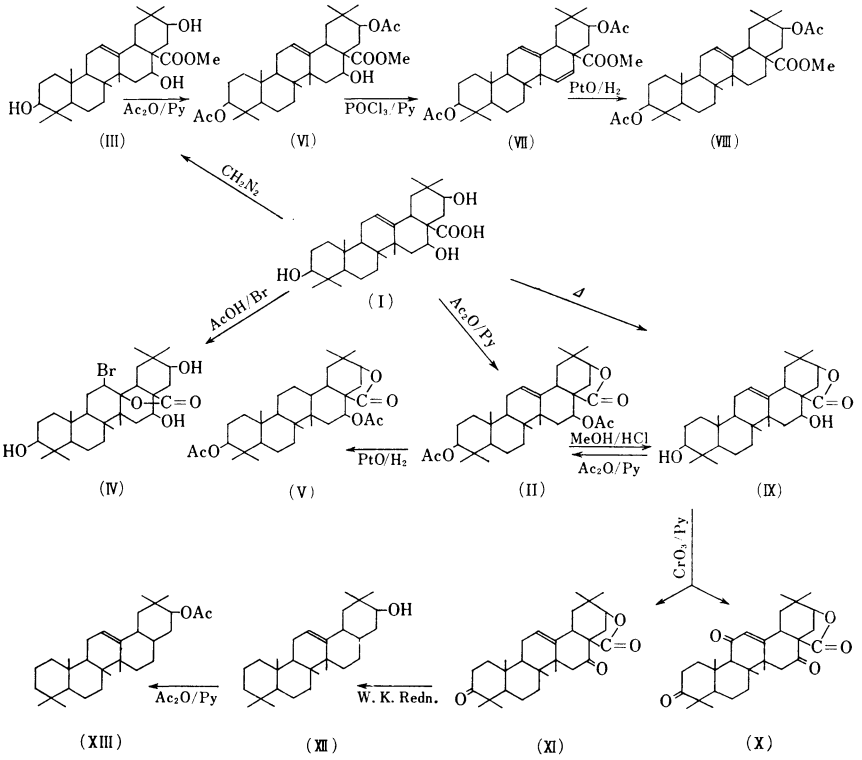
6) I. P. Varshney, *Proc. Nat. Acad. Sci., India, Sect. A* **XXXV**, 418 (1965).

7) I. P. Varshney and M. S. Y. Khan, *J. Pharm. Sci.*, **50**, 923, (1961); This Bulletin, **38**, 1214 (1965).

8) Z. Naim, Ph. D. thesis, Aligarh Muslim University, 1961.

9) I. P. Varshney, Intern. Sympo. Natural Products, I. U. P. A. C., Bruxelles, 1962, Resume des Communications.

10) I. P. Varshney, *Indian J. Chem.*, **7**, 446 (1969).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COOMe
OH
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Ac₂O/Py
AcO
OAc
POCl₃/Py
PtO/H₂
(III)
(VI)
(VII)
(VIII)
CH₂N₂
COOH
(I)
AcOH/Br
Δ
Br
C=O
(IV)
(V)
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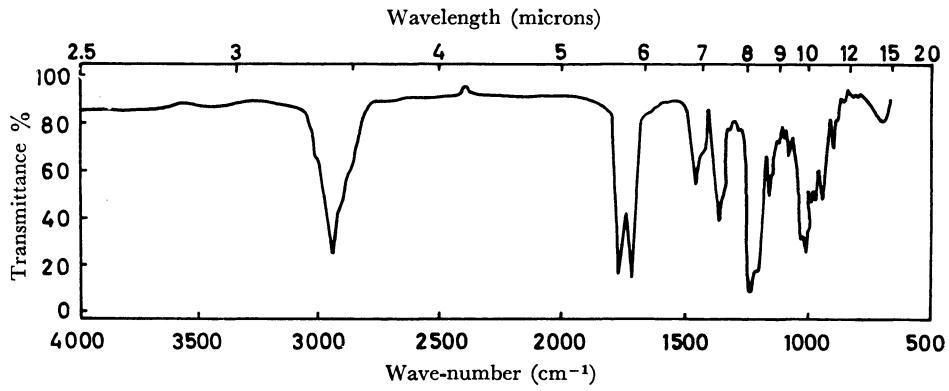
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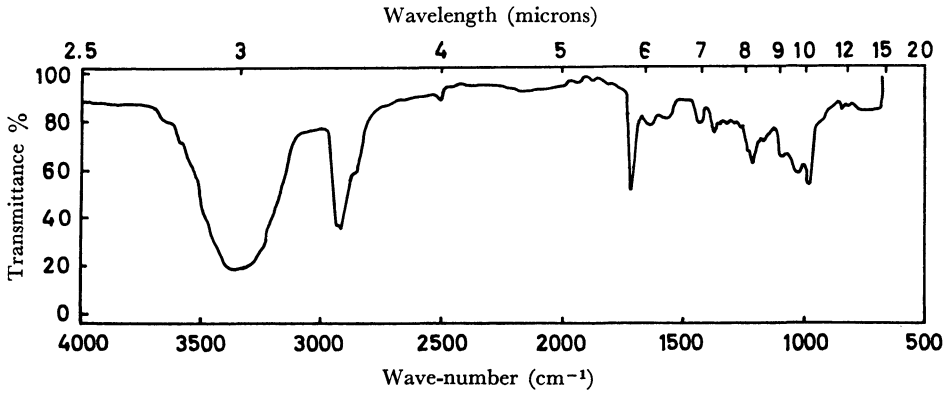
Fig. 1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Wave-number (cm⁻¹)

Fig. 2

mass spectrum of the compound showed its molecular weight as 554 corresponding to the formula $C_{34}H_{50}O_6$. The general fragmentation pattern was that associated with triterpenes.

The acetate on deacetylation gave colourless needles of the free genin (I) mp 278—282°C. The genin on methylation with diazomethane gave a methyl ester (III) as colourless needles mp 224—225°C. Acetylation of the methyl ester (III) gave diacetate methyl ester (VI) as colourless needles mp 203—205°C. All these compounds gave yellow colour with tetranitromethane. All these evidences and analytical results showed that acacic acid is a trihydroxy-monocarboxylic acid with molecular formula $C_{30}H_{48}O_5$ corresponding to pentacyclic triterpenes.

The relationship of acacic acid to β-amyrin group was fixed by the facile formation of a saturated bromolactone*4(IV) mp 259—262°C, by the action of bromine on acacic acid in acetic acid. Formation of a bromolactone[11] not only indicated an olean type skeleton, but also fixed the position of the carboxyl group at C-28 and the double bond between carbon 12—13.

The infrared spectrum of acacic acid methyl ester in pyridine (Fig. 2), taken under prescribed conditions,[12] through the courtesy of Prof. R. Tschesche, showed only two absorptions at 1380 and 1355 cm^{-1} in region 'A' and three absorptions at 1330, 1300 and 1267 cm^{-1} in region 'B' which clearly indicated its relation to the β-amyrin group.

Furthermore, the relation of acacic acid to the β-amyrin group was fixed by the formation of the (di)acetate methyl ester of the hydrolysis product of sapogenin B of Styphnodendron coriaceum[13] (Proceric acid[14]) (VIII) from acacic acid (I). Acacic acid (I) on methylation with diazomethane gives a methyl ester (III) which on acetylation with acetic anhydride and pyridine in the cold yielded a (di)acetate methyl ester (VI) leaving one of the hydroxyl groups free. This hydroxy acetate methyl ester (VI) on dehydration with phosphorous oxychloride in pyridine gave a diene (VII) (*cf.* Varshney *et al.*,[15] echinocystic acid diene), which gives a very deep colour with tetranitromethane. Diene (VII) on hydrogenation with Adams' platinum yielded a product (VIII) which has been identified as proceric acid (di) acetate methyl ester[14] (diacetate methyl ester of the hydrolysis product of sapogenin B of Styphnodendron coriaceum[13]).

The formation of a diacetate lactone (28→21) (II) by acetylation of acacic acid (I) suggested that one of the hydroxyl groups should be situated at a position γ- to the carboxyl group, *i.e.* at C–15, 19 or 21. The above conversion of acacic acid into a compound of known structure confirmed that the position of the hydroxyl group is 21.

This conversion of acacic acid (I) to a compound of known structure (VIII) established not only the relation of acacic acid to the β-amyrin group but also established that one of the hydroxyl groups in acacic acid (I) is located at C–3, another at C–21, and the carboxyl at C–28.

The formation of the diacetate methyl ester (VI) instead of the expected triacetate on acetylation of the methyl ester paralleling the formation of a monoacetate of methyl echinocystate[15] suggested the possible location of the third hydroxyl group at a position β- to the carboxyl group (*i.e.* on C-16 or C-22), the methylation of which hinders the formation of acetate derivatives.

Methyl ester (III) on periodic oxidation was recovered unchanged, indicating the absence of a 1,2-diol system in the compound. Similarly the methyl ester failed to form an isopropylidene derivative, which while confirming the above observation also indicated the absence of a *cis*-1, 3-diol system (*cf.* Tomentosic acid[16]), thus eliminating the adjacent position (*i.e.* C-22) for the third hydroxyl group, leaving only position C-16 for the third hydroxyl group.

Acacic acid diacetate lactone (II) undergoes hydrogenation at atmospheric pressure in the presence of Adams, catalyst, yielding a saturated compound (V), contrary to the normal behaviour of the members belonging to the β-amyrin group. This abnormal behaviour can only be explained on the basis of some conformational or stereochemical arrangements differing from those accepted. This also suggests the possibility of the location of one hydroxyl group in a γ-position to the carboxyl group (*i.e.* position 21). The diacetate lactone (II) on partial hydrolysis with potassium carbonate in methyl alcohol gave a product mp 307—320°C which could not be characterised. The diacetate lactone (II) on hydrolysis with methanolic hydrochloric acid (10%) gave colourless needles of hydroxy lactone (IX) mp 252—

*4 The purity of the bromolactone (IV) is doubtful but the product obtained does not show any unsaturation with tetranitromethane, indicating that the double bond has been removed by lactone formation.

11) M. Shamma, *Drug Standards*, **27**, 42 (1959).

12) G. Snatzke, F. Lampert and R. Tschesche, *Tetrahedron*, **18**, 1417 (1962).

13) B. Tursch, E. Tursch, I. T. Harrison, Gloria Berenica Chagas Tolentino De Calvalhe Brazao da Silva, H. J. Moterio, B. Gilbert, B. Mors Walter and C. Djerassi, *J. Org. Chem.*, **28**, 2390 (1963).

14) I. P. Varshney and S. Y. Khan, *J. Pharm. Sci.*, **53**, 1532 (1964); I. P. Varshney, *Bull. Chem. of Terpenoids, Natl. Inst. Sci. India, New Delhi*, No. 37, 95—99 (1968).

15) Ch. Sannie, H. Lapin and I. P. Varshney, *Bull. Soc. Chim. Fr.*, **1957**, 1440; I. P. Varshney, Thesis, Dr. es Sc., Univ. Paris, Soutenue le 21 Decembre, 1956.

16) L. R. Row and L. S. R. S. Rao, *Tetrahedron*, **18**, 827 (1962).

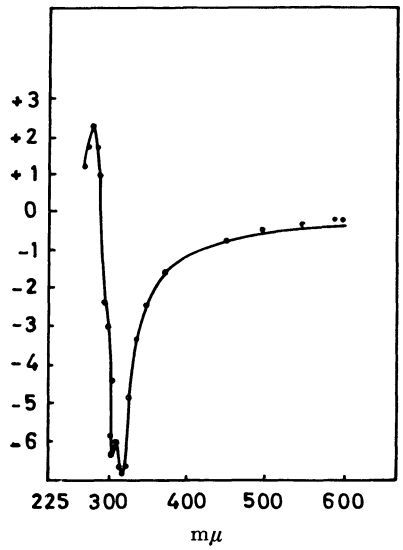


Fig. 3

254°C, which was confirmed by its reacetylation to diacetate lactone (II). Pyrolysis of acacic acid (I) also resulted in the formation of the same dihydroxy lactone (IX). The ORD curve (Fig. 3) of the diketo lactone (XI) formed by the oxidation of dihydroxy lactone (IX) with chromic acid in the cold shows a strong negative multiple Cotton effect, closely resembling that of *nor*-echinocystenolone acetate.[17] This shows that the stereochemical arrangement in acacic acid is iden-

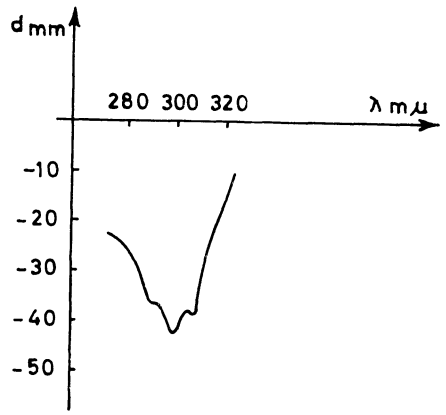
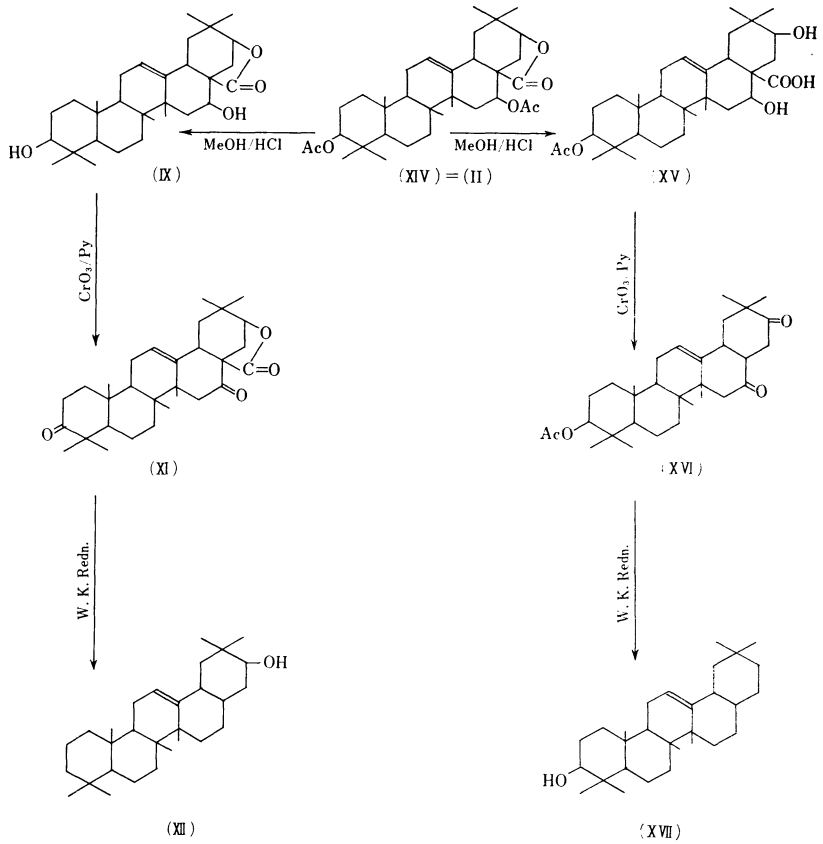


Fig. 4

$\Delta\varepsilon$	309	−2.02
	298	−2.29
	291—292	−1.97



17) C. Djerassi, J. Osiecki and W. Closson, *J. Amer. Chem. Soc.*, **81**, 4587 (1959).

tical with that of *nor*-echiyocystenolone acetate (D-E *cis* fusion). Thus DE *cis* ring junction was assigned to acacic acid. A *trans* fusion should be expected to give a positive Cotton effect. The CD curve (Fig. 4) also supported this finding.

Ketone (XI) on Wolff-Kishner reduction gave colourless needles mp 183—188°C (XII) a *nor*-compound by decarboxylation of the β-keto acid formed under the reaction conditions. This compound on acetylation gave an acetate mp 210—216°C (XIII). The infrared spectrum of compound (XII) showed a strong band in the hydroxyl region. The analytical results correspond to the formula $C_{29}H_{48}O$, and thus this compound should have the structure (XII) on the basis of the scheme outlined.

Earlier, the product (XII) mistakenly thought to be 28 -*nor*-β-amyrin (XVII) according to the scheme shown below (XIV—XVII) as no depression in mp took place when the compound (XII) was mixed with an authentic sample of *nor*-β-amyrin (XVII).[18] But later infrared studies showed that this compound was different from 28-*nor*-β-amyrin. This led to the complete revision of the scheme (XIV—IX—XII). More extensive study of compound (IX) was done through IR, NMR, and mass spectrometry to give structure (IX).

It may be pointed out that compound (XII) and 28-*nor*-β-amyrin (XVII) have given the same R_f values on TLC, and have the same elemental composition. However there is a large difference in the IR spectra of the two compounds.

The formation of *nor*-compound (XII) starting with C-30 ketone (XI) definitely indicated that the ketone was a β-ketolactone, as only this hypothesis can explain the decarboxylation during Wolff-Kishner reduction due to the formation of a β-keto acid under the alkaline conditions of the reaction. All evidences fixed the position of the third hydroxyl group to be C-16. The formation of the acetate methyl ester of the sapogenin B of Styphnodendron coriaceum coupled with other evidences discussed above fixed the structure of acacic acid as 3,16,21-trihydroxyolean-12-en-28-oic acid (I) leaving the question of stereochemistry and conformation undecided.

The NMR spectra of acacic acid diacetate lactone(II) (Fig. 5) showed signals corresponding to the lactonic proton at 4.2 ppm as a doublet. This is quite in agreement with the structure formulated, for the dihedral angle between the hydrogen at C-21 and one of the hydrogens at C-22 is about 90° which leads to a doublet rather than the triplet normally obtained by splitting the vicinal hydrogens. The acetoxyl proton on C-3 appears in the form of quadruplet centered at 4.5 ppm; $J_1=9$ Hz and $J_2=2$ Hz which indicates that the hydrogen is α- and the hydroxyl β-oriented.

The other acetoxyl proton at C-16 appears in the form of a quadruplet centered at 5.0 ppm; $J_1=12$ Hz and $J_2=5$ Hz also indicating a β-orientation for the hydroxyl group at C-16.

The hydroxyl group on C-21, on the basis of lactone formation, can be designated as β. The α-hydroxyl group will not form a lactone with the β-carboxyl group at C-28.

Therefore acacic acid(I) is 3β,16β,21β-trihydroxy-18β-olean-12-en-28-oic acid.

The NMR spectra of compound (IX)(Fig. 6) showed signals corresponding to vinyl proton at 5.38 ppm as a triplet characteristic of the proton of the β-amyrin group. One proton appearing at 4.2 ppm as a doublet should be a lactonic proton (CH–O–C=O). Two hydroxyl protons appeared at 1.53 ppm as singlets.

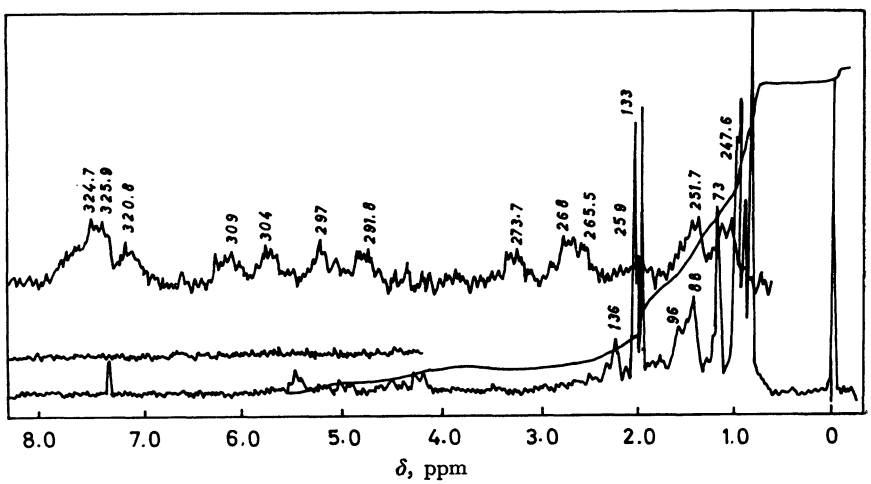


Fig. 5

18) I. P. Varshney and K. M. Shamsuddin, *Tetrahedron Lett.*, **1964**, 2055.

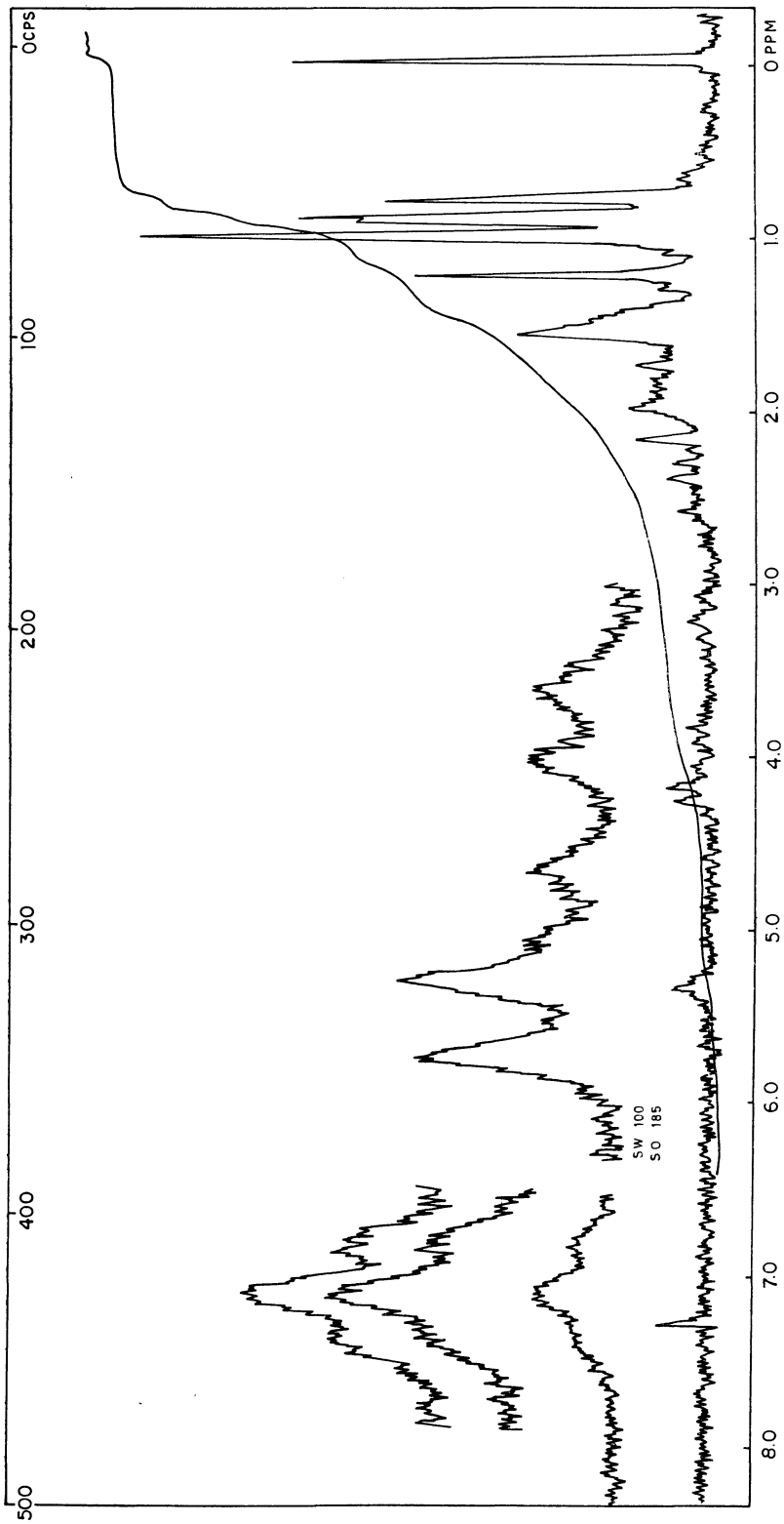


Fig. 6

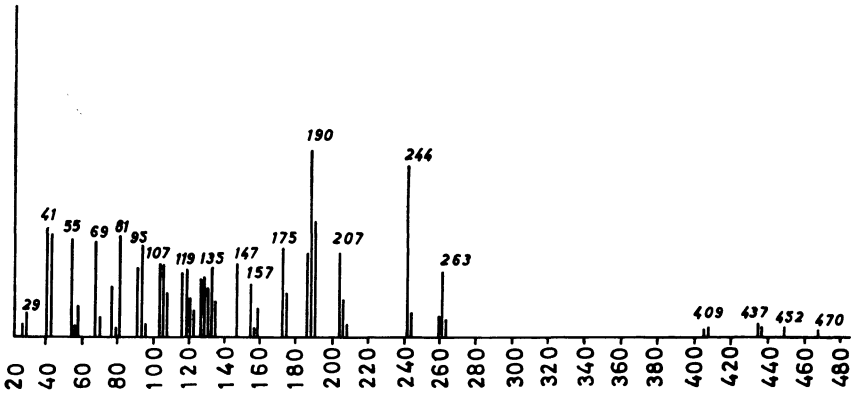
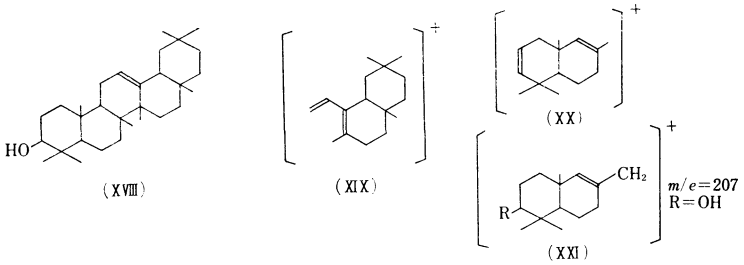
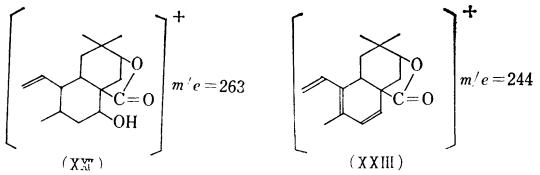


Fig. 7



The mass spectrum (Fig. 7) of (IX) had the M^+ peak at 470 which is in definite agreement with the proposed structure, $C_{30}H_{46}O_4$. There is a peak at *m/e* 452 arising out of the loss of one molecule of water. In the triterpenes Δ^{12}-compounds (XVIII) undergo predominantly retro-Diels-Alder reaction leading to fragments (XIX) and (XX) while the rings A and B lead to the fragments (XXI).[19]

Thus the peaks at *m/e* 263 and 244 are due to fragments (XXII) and (XXIII) which correspond to fragment (XIX). The other peak at *m/e* 207 is due to fragment (XXI; R–OH). The peak at *m/e* 175 can be attributed to the *m/e* 190 fragment minus a methyl group.



The oxidation of dihydroxy lactone (IX) with pyridine-chromium trioxide complex, gave a product (XI) mp 338—342°C. It gave a yellow colour with tetranitromethane and formed a semicarbazone mp 278—282°C.

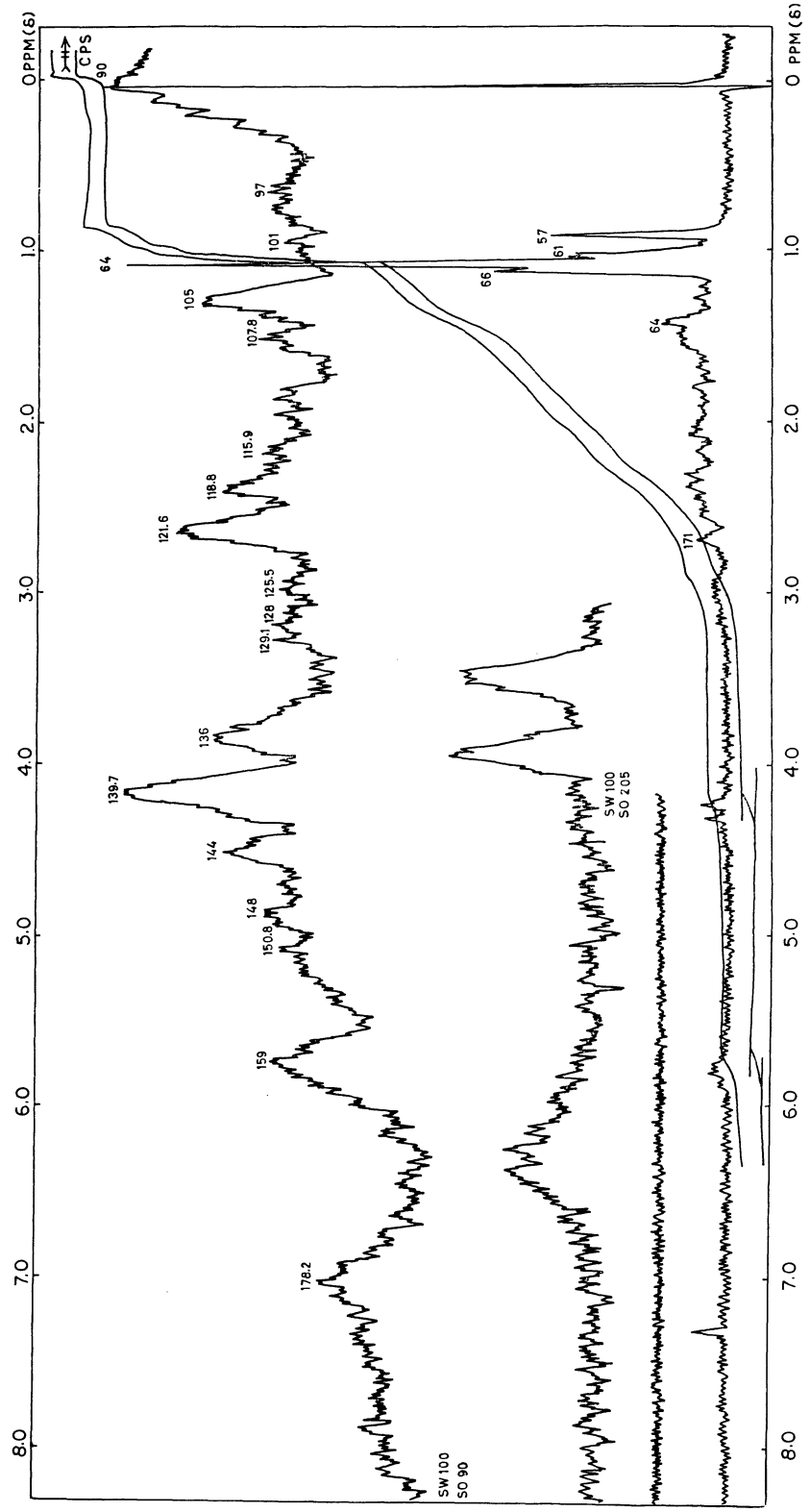
The NMR spectrum (Fig. 8) of compound (XI) shows signals of a proton at 4.2 ppm, as a doublet which is due to the lactonic proton as in the case of (IX) (Fig. 6). The spectrum also shows the presence of the vinylic proton at 5.8 ppm.

The mass spectrum (Fig. 9) of this compound also confirms this postulation. It has M^+ peak at 466 which is in entire agreement with the formula $C_{30}H_{42}O_4$ for the diketolactone. The peak at *m/e* 451 should correspond to M-15, due to the loss of a methyl group. The peak at *m/e* 260 is due to the normal fragmentation procedure and corresponds to the 'a' type fragment[13] represented in this case by (XXV), as in the case of sapogenin B of Stryphnodendron coriaceum (XXIV) which has also a lactone function between the carboxyl at C-28 and the hydroxyl at C-21 leading to fragment (XXVII). This compound also gives rise to fragment (XXVI) corresponding to *m/e* 215.

There is a peak at *m/e* 480 which has been assumed to be due to a small quantity of the triketo product (X) formed by allylic oxidation of the dihydroxy lactone (IX) and whose molecular formula is $C_{30}H_{40}O_5$. The presence of traces of the triketo product (X) was detected by thin layer chromatography. A weak peak at *m/e* 315 in the mass spectrum can be attributed to the fragment (XXVIII) arising out of the 11-keto compound (X).

Usually pentacyclic triterpenes of the β-amyrin group have rings A-B, B-C, and C-D *trans* and D-E *cis* fused, with an all chair conformation.

19) H. Budzikiewicz, J. J. M. Wilson and C. Djerassi, *J. Amer. Chem. Soc.*, **85**, 3688 (1963).


OPPM(δ)
CPS
90
64
97
101
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107.8
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125.5
128
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136
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144
148
150.8
159
178.2
SW 100
SO 90
SW 100
SO 205
57
61
66
64
171
O PPM(δ)
1.0
2.0
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4.0
5.0
6.0
7.0
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Fig. 8

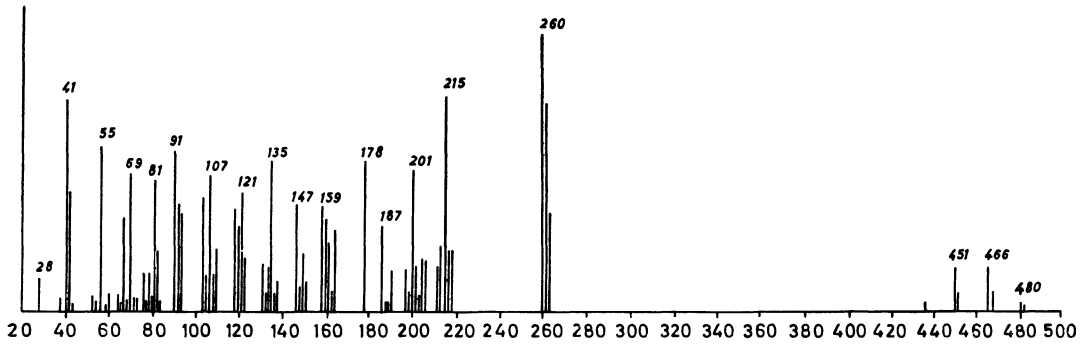
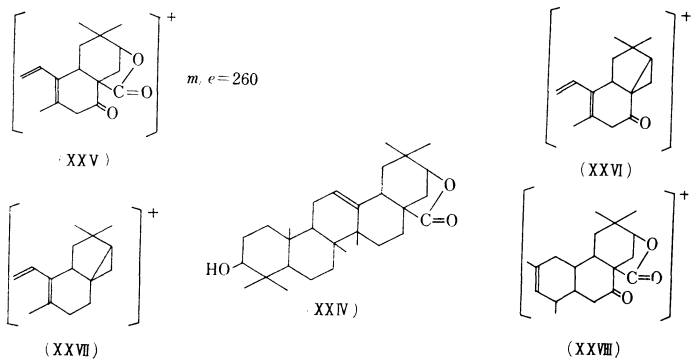


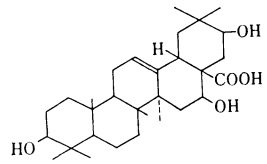
Fig. 9



Easy hydrogenation of the diacetate lactone(II) of acacic acid is contrary to the properties of β-amyrin group which cannot be hydrogenated under normal conditions,[11,13] and therefore indicates that some subtle conformational difference exists in this compound. These instances are parallel to the behaviour of sapogenin B of Stryphnodendron coriaceum,[13] and can be explained on the basis that ring E exists in boat form and consequently ring D in a *quasi-boat* form.[13] The proximity of the C-21 hydroxyl and the C-28 carboxyl groups which tends to make the lactonisation easy can be seen by the study of a molecular model of acacic acid. Such a lactonisation could also be possible with a *trans* D-E fusion but this possibility is eliminated on the basis of the ORD (Fig. 3) and circular dichroism (Fig. 4) curves of the diketolactone(XI) which strongly resemble that of *nor*-echinocystenolone acetate.[17] The latter has a ring D-E *cis* fusion, and this resemblance indicates identical stereochemical arrangement around ring D in both the compounds. Thus D-E ring junction is *cis* in case of acacic acid(I). As this assignment made by Djerassi *et al.*[17] is accepted for *nor*-echinocystenolone, acacic acid(I) has an 18β-H configuration, except for the improbable event of an erroneous assignment of configuration to *nor*-echinocystenolone.[17] This would not change the conclusions regarding the stereochemistry except as regards configuration at C-18, in acacic acid.

While acacic acid(I) itself could exist in all chair conformation, this is impossible for the 28—21 lactone(II) of this series. A study of models shows that such a lactone can be easily formed provided ring E is constrained into a boat form. Ring D in the lactone can then adopt several conformations, but one of them, a twist form, appears to minimize all interactions effectively. In this conformation the 16β bond is *quasi*-equatorial and the geometrical relationship between the 16α-H and the 15-CH_2 group is such that the observed coupling constants are explained.

Therefore acacic acid can now be represented as 3β,16β,21β-trihydroxy-18β-olean-12-en-28-oic acid, and in its diacetate lactone (28—21), the rings E and D are in *boat* and *quasi-boat* forms respectively (XXIX).



(XXIX)

Experimental*5

Extraction of Saponin. The pods of *Acacia concinna* DC. (900 g) obtained from Kerala (South India) broken into small pieces were exhaustively extracted with ethanol under reflux and solvent recovered. This left a dark brown syrupy residue which was successively treated with ether, petroleum ether, carbon tetrachloride, chloroform, and acetone. The mass left over was dissolved in a small quantity of alcohol, and saponin was precipitated by addition to a large quantity of ether/acetone. This process was repeated several times followed by treatment with activated charcoal to give a fairly pure specimen of saponin (28 g).

Hydrolysis of Saponin. Saponin (10 g) was dissolved in a large volume of water and heated with sulphuric acid (10%) on a water bath for an hour followed by refluxing for another hour. Sapogenin was filtered, and dried (7.5 g).

Purification of Sapogenin. Crude sapogenin (7.5 g) was refluxed with alcoholic potassium hydroxide (10%; 600 cc) for one hour and the volume reduced to half. The solution was then diluted with water (2 *l*) and extracted with ether. The aqueous layer was then acidified with hydrochloric acid and the precipitated acid genin filtered, washed and dried (3.2 g).

Diacetate Lactone (II). Genin (5 g) was acetylated in the usual manner by heating with acetic anhydride and pyridine. The product was crystallized from methanol mp 235—236°C. It gave a positive colour with tetranitromethane. Genin was acetylated by refluxing with acetic anhydride and sodium acetate as well as with acetic anhydride and pyridine in the cold gave the same product mp 235—236°C. It showed no depression in mp when mixed with an authentic sample of acacic acid diacetate lactone obtained from Acacia intsia bark.

Acacic Acid (I). Acetate (II) was refluxed with alcoholic potassium hydroxide (300 cc; 10%) for two hours and the volume reduced to half. The solution was poured into excess of water and acidified with hydrochloric acid which precipitated genin. The precipitated sapogenin was filtered, washed and crystallized from a large volume of methanol mp 278—282°C. It gave yellow colour with tetranitromethane. Mixed mp with authentic sample of acacic acid obtained from Acacia intsia bark showed no depression.

Bromolactone (IV). A suspension of acacic acid (I) (500 mg) in acetic acid (50 cc) containing anhydrous sodium acetate (2 g) was stirred during addition of bromine (1 cc) in acetic acid (25 cc) for an hour and the contents diluted with water. The product was extracted with ether and the water extract washed with sodium thiosulphate, sodium bicarbonate and water. Recovery of ether furnished the bromolactone which was crystallized from methanol, mp 259—262°C (*cf.* *4). It gave no colour with tetranitromethane.

Methyl Ester(III). Acacic acid (I) was treated with an excess of ethereal solution of diazomethane and the reaction mixture was kept overnight at room temperature. The product isolated by the evaporation of ether was crystallized from methanol mp 224—225°C. It gave a yellow colour with tetranitromethane. Mixed mp with an authentic sample was undepressed.

Methyl Ester Diacetate (VI). The methyl ester (1 g) (III) was acetylated with acetic anhydride (100 cc) and pyridine (100 cc) in the cold in the usual manner. It was crystallized from methanol as needles mp 203—205°C. It gave positive colour with tetranitromethane. Mixed mp with authentic sample was undepressed.

Found, C, 71.46, 71.36; H; 9.64, 9.50%. Calcd for $C_{35}H_{54}O_7$: C, 71.64; H, 9.28%.

Periodic Oxidation of Methyl Ester (III). The methyl ester (III) (500 mg) was dissolved in ethanol (95%; 30 cc) and treated with a solution of sodium periodate (150 mg) in sulphuric acid (6 cc). The mixture was kept at room temperature for 18 hours and then poured into excess of water. The precipitate was filtered, washed and crystallized from methanol as colourless needles mp 223—224°C. The mixed mp with the starting material was undepressed.

Isopropylidene Derivative of Methyl Ester. The methyl ester (III) (500 mg) in dry acetone (25 cc) was treated with a drop of concd. hydrochloric acid. It did not give any precipitate. The solvent was then removed and the product left over was crystallized from methanol mp 223—224°C. The mixed mp with the starting material showed no depression.

Dehydration of VI. The diacetate methyl ester (VI) (300 mg) was refluxed with pyridine (10 cc) and phosphorous oxychloride (2 cc) for 5 hr. It was then diluted with ice cold water. The product was extracted with ether, and the ethereal extracts washed with water. Evaporation of ether and crystallization from methanol furnished compound (VII) mp 280—282°C. It gave a deep yellow colour with tetranitromethane.

Found: C, 73.54, 73.78; H, 9.65, 9.19%. Calcd for $C_{35}H_{52}O_6$: C, 73.91; H, 9.22%.

Hydrogenation of VII. The dehydration product (VII) (200 mg) was dissolved in acetic acid (50 cc) and hydrogenated at atmospheric pressure and room temperature in the presence of Adams' catalyst. The product was crystallized from methanol as colourless shining needles mp 266—270°C. It still gave positive reaction with tetranitromethane.

Found: C, 72.88, 72.76; H, 9.24, 9.15%. Calcd for $C_{35}H_{54}O_6$: C, 73.64; H, 9.54%.

Hydrogenation of II. The diacetate lactone (II) (100 mg) was hydrogenated in acetic acid (20 cc) in the presence of Adams' catalyst at room temperature and pressure. It was crystallized from methanol in the form of colourless plates mp 280—285°C (V). It did not give any colour with tetranitromethane.

Found: C, 72.73, 72.50; H, 9.78, 9.90%. Calcd for $C_{34}H_{52}O_6$: C, 73.34; H, 9.41%.

Partial Hydrolysis of II. The diacetate lactone (II) (500 mg) was refluxed with methanol (50 cc) containing hydrochloric acid (10 cc) for two hours and the product worked out in the usual manner. It was crystallized from methanol as colourless crystals mp 252—254°C (IX). It gave a yellow colour with tetra-

*5 All the melting points recorded in this paper have been taken on Koflers hot microscopical melting point apparatus and are corrected. NMR spectra have been taken on varian A-60 and CD on Roussel Jouan Dichrograph. IR spectra have been recorded on Perkin-Elmer models 137, 21, 221 and Beckman IR5A.

nitromethane.

Acetylation of IX. The partial hydrolysis product (IX) (200 mg) was acetylated in the cold with pyridine (10 cc) and acetic anhydride (10 cc) in the usual manner and crystallized from methanol mp 234—236°C. Mixed mp with diacetate lactone (II) showed no depression.

Pyrolysis of I. Acacic acid (I) (150 mg) was taken in a pyrex tube and heated in an oil bath at 290—300°C for 25 min. The melt obtained was extracted and crystallized from methanol 252—254°C. It gave no depression on mixed melting with dihydroxy lactone (IX).

Oxidation of IX. A solution of dihydroxy lactone (IX) (200 mg) in pyridine (5 cc) was added to a suspension of chromium trioxide pyridine complex prepared from chromium trioxide (300 mg) and pyridine (8 cc) and the mixture kept at room temperature for 24 hours after which it was poured into crushed ice. The product worked out in the usual manner was crystallized from methanol-chloroform mixture mp 338—342°C (XI). It gave yellow colour with tetranitromethane. This product formed a semicarbazone mp 278—282°C.

Thin layer chromatography of the ketone using cyclohexane; ethylacetate system showed two spots, one major and one minor, the latter with a faster running rate. The product was purified by preparative thin layer chromatography and the pure ketone (XI) used for NMR measurements. Mass spectrometry was carried out with untreated product which clearly showed the impurity.

Wolff-Kishner Reduction of XI. Ketone (XI) (100 mg) was dissolved in diethylene glycol (20 cc) containing metallic sodium (400 mg) and to it was added hydrazine hydrate (100%). Then the reaction mixture boiled at 180°C. It was refluxed at this temperature for 18 hours after which hydrazine hydrate was evaporated till the solution boiled at 210°C. Refluxing was continued for another 24 hr and the reaction mixture worked out in the usual manner. No acidic product could be isolated, and the neutral product was crystallized from methanol-ethyl acetate mixture; mp 183—188°C (XII). It gave a positive colour with tetranitromethane.

Found: C, 84.20; H, 11.73%. Calcd for $C_{29}H_{48}O$: C, 84.40; H, 11.73%.

Acetylation of XII. The reduction product (XII) (60 mg) was acetylated with pyridine and acetic anhydride in the usual manner. The acetate was crystallized from ethyl acetate methanol mixture; mp 210—216°C (XIII). It gave positive colour with tetranitromethane.

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