PART - I

PARTIAL SYNTHESIS OF DIMETHYL DIHYDRO-GEANOTHATE STARTING FROM BETULINIC ACID

PART - 1

CHAPTER - 1

A short review on the isolation, structure and stereochemistry of ceanothic acid :

Section A : Isolation :

Ceanothic acid was originally isolated by Julian, Pikl and Dawson[1] from the alcohol extract of the root bark of *Ceanothus americanus* and has since been isolated from a number of Australian Rhamnaceae species[2,3]. This substance was characterized by Julian *et al*[1] as a hydroxy dicarboxylic acid and attributed the formula $C_{29}H_{44}O_5$. It was characterized by the preparation of a dimethyl ester and of a dimethyl ester acetate. In 1958 Boyer *et al*[2] isolated emmolic acid from *Emmenospermum alphitonioides F.Muell* (Rhamnaceae). This acid was later found to be identical with ceanothic acid by Birch and coworkers[4]. de Mayo and Starratt[5] in 1962 also isolated ceanothic acid from *Ceanothus americanus* but their method of isolation was not same as that used by Julian, Pikl and Dawson[1]. They could not successfully isolate the acid by adopting the procedure used by Julian *et al*[1]. de Mayo and Starratt[5] remarked on the variability of the plant and subsequent workers[6] have also made similar observations. By adopting different procedures, de Mayo *et al*[5] were able to isolate different proportions of the various

acid constituents. The method leading to isolation of ceanothic acid is described here. The ground root bark of Ceanothus americanus was extracted continuously with ether in a Soxhlet apparatus for 33 hours. The residue was extracted with light petroleum under reflux for 3 hours, the light petroleum then being replaced, and the process repeated four times. The residue in ethereal solution, was extracted exhaustively with 2% potassium hydroxide solution. A solid, which separated at the interface, was removed by filtration. Acidification of the alkaline solution and isolation with ether gave the crude acid mixture which was further defatted by extraction with light petroleum. The acid mixture was added, in benzene solution, to a column of silica. Elution with benzene-ether (20:1) gave an acid, m.p. 350-54° (decomp.), $(\alpha)_D$ 39° (C, 1·23 ; pyridine). Further elution with the same solvent mixture then gave betulinic acid, m.p. 270-85°. Further elution with benzene-ether (10:1) then gave material which after two crystallisations from ether-methanol gave pure ceanothic acid, m.p. 356-57° (gas evolution) (lit.[1] 354°), ν_{max}^{nujol} 3480, 1720, 1641 and 883 cm^{-1}.

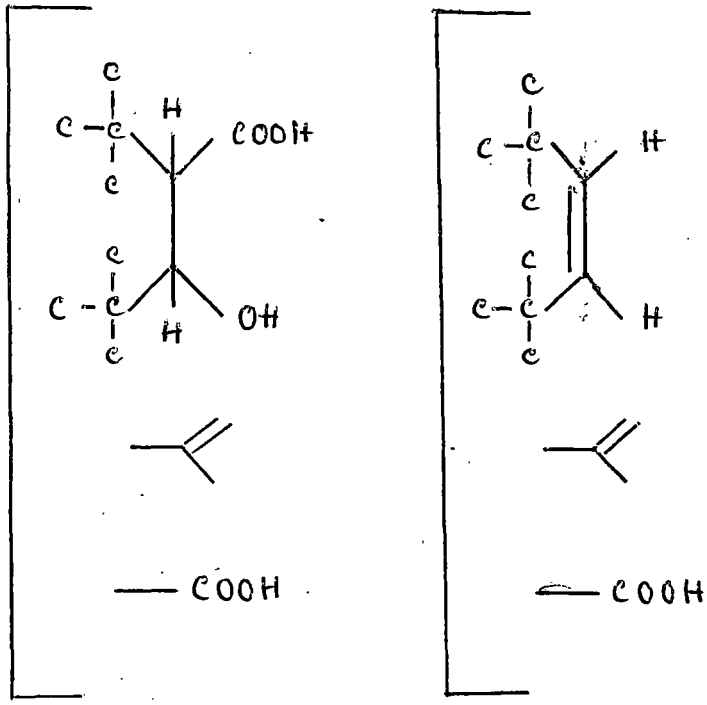
Section B : Structure of Ceanothic acid :

The systematic study for the determination of the structure of ceanothic acid was mostly due to de Mayo and Starratt[5].

Their work is summarised below.

The infrared spectra of ceanothic acid and of its dimethyl ester showed bands ν_{max}^{nujol} 833 and ν_{max}^{nujol} 889 cm^{-1} respectively. These bands indicated the presence of an exo methylene group. This interpretation was supported by the disappearance of this band on catalytic hydrogenation both of the acid and of the ester to their respective saturated dihydro compounds. The NMR spectrum of the ester showed a doublet at τ 5·37 (J~7·7 cps) (2 hydrogens) attributed to the methylene group and a singlet (3 hydrogens) at τ 8·36 attributed to vinylic methyl group[7]. These observations, in the absence of any other double bond, indicated the presence of an isopropenyl group. The NMR spectrum, further, showed singlets (1 hydrogen) at τ 5·98 and τ 7·51, indicating that both the hydroxyl group and one of the carboxyl groups were attached to carbon atoms bearing one hydrogen atom. The above bands are in appropriate position for the respective methine hydrogens[7]. Evidence for the proximity of the hydroxyl & group and the secondary carboxyl group was obtained by an examination of the "lactone" obtained by Julian, Pikl and Dawson[1]. This substance was shown to be an unsaturated acid by its conversion, with diazomethane, to the corresponding ester. In addition to the bands at τ 5·40 and τ 8·46 in the NMR spectrum, indicative of the continuing presence of the isopropenyl group, two hydrogens producing an AB pattern (doublets at τ 4·16 and τ 4·66,

$J_{AB} \sim 5{\cdot}4$ cps) were present. The formation of this pattern was due to the conversion of 1 to 2, that is, the dehydration-decarboxylation of a β-hydroxy acid.



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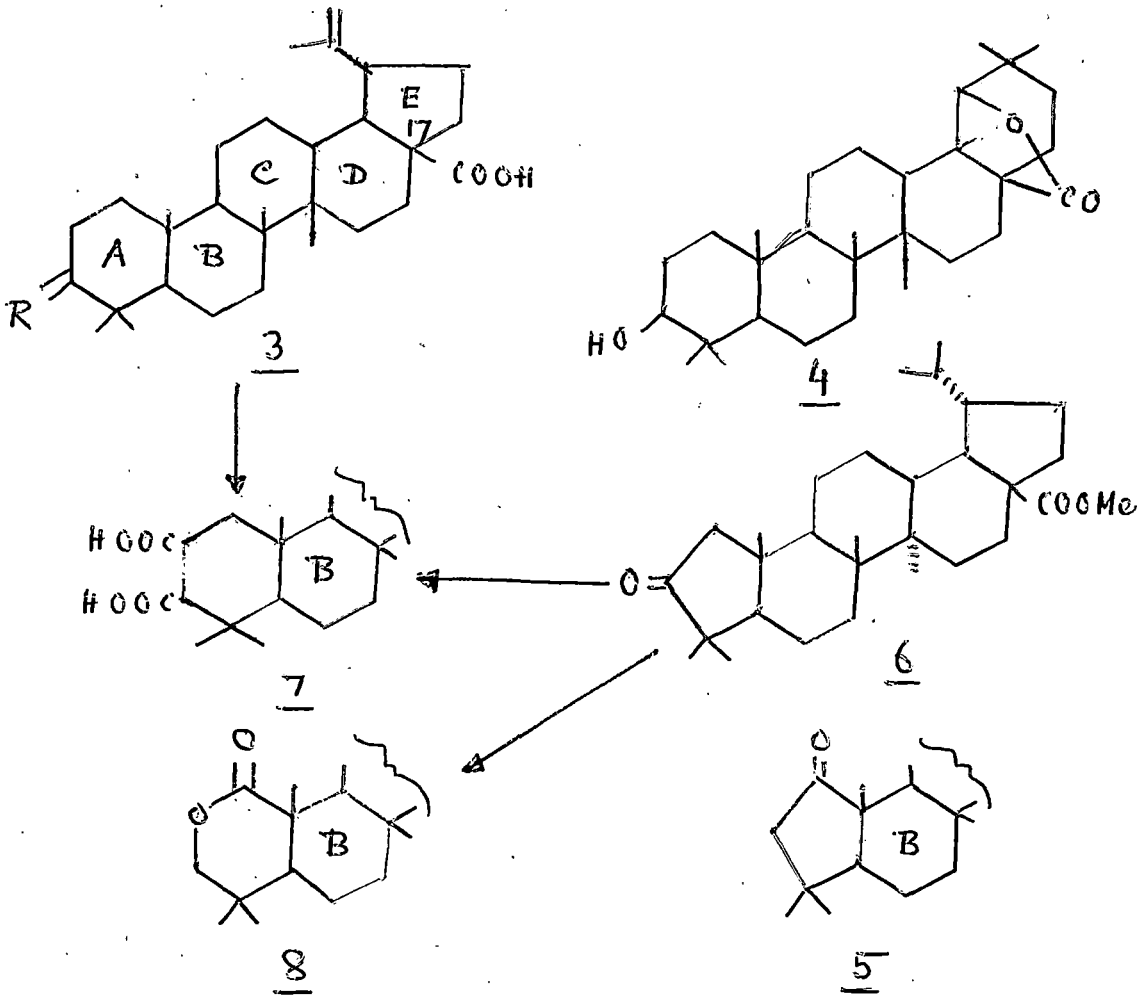
de Mayo et al confirmed this on the basis of the following experiments. Dimethyl ceanothate was converted into the benzoate which was isolated as an oil and the latter was pyrolysed at 330° with the elimination of benzoic acid. The isolated anhydro-acid showed the expected ultraviolet end absorption for an isolated isopropenyl group and an $\alpha\beta$-unsaturated ester. Furthermore, a band (singlet, 1 hydrogen) at τ 3·9 in the NMR spectrum

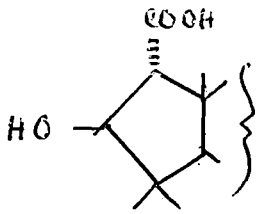
indicated the presence of the expected vinyl hydrogen in addition to those of the isopropenyl group[7]. Since dihydroceanothic acid and its dimethyl ester showed no absorption in the ultraviolet spectrum in the 200-210 mμ region, they were, presumably saturated. It followed that ceanothic acid was, therefore, pentacarbocyclic. They assumed that ceanothic acid was probably related to the lupeol-betulin-betulinic acid (3 , R=H,OH) in view of the presence of the isopropenyl group and this view was strengthened by the presence of betulinic acid itself amongst the root constituents. One of the characteristic transformations of lupeol-betulin group of substances is the ready acid catalysed expansion of the terminal E-ring to give derivatives of the β -amyrin series[8]. In those substances having a carboxyl function at C_{17} concomitant lactonisation occurs ; betulinic acid (3, R = H,OH) for instance, is converted into 4 . When subjected to suitable conditions - refluxing formic acid for 3 hours - ceanothic acid was converted into a γ -lactone, ν_{max} 1696 (carboxyl) and 1762 cm^{-1} (γ-lactone) with the simultaneous disappearance of the isopropenyl group. The resulting monocarboxylic acid lactone was further characterized as the acetate, indicating the non-participation of the hydroxyl group in the lactonisation process, and as the methyl ester. In order to accommodate the functional groups indicated in 1 , they suggested that some

modification of ring A of betulinic acid was necessary. The grouping in 1 was suggestive of the occurrence of a 'biogenetic' pinacolic rearrangement at some stage in the genesis of ceanothic acid, such as may take place in the formation of gibberellic acid[9] and of the aldehyde in magnamycin[10]. Oxidation of methyl dihydroceanothate with sodium dichromate gave the corresponding ketone. Alkaline hydrolysis afforded a ketonic monoester with the loss of carbon dioxide expected of a β-keto ester. The substance showed an unresolved band in the infrared spectrum, ν_{max} 1738 cm^{-1} for the cyclopentanone and ester, while its precursor showed bands at $\nu_{max}^{CCl_4}$ 1750 (cyclopentanone) and $\nu_{max}^{CCl_4}$ 1727 cm^{-1} (ester) ([redacted]).

From the above experiments de Mayo *et al* proposed two structures 5 and 6 for the ketone, but its properties suggested that it was 6, a substance previously prepared by Ruzicka *et al*[11,12] from betulonic acid by hydrogenation, nitric acid oxidation to 7, followed by pyrolysis and esterification.



Direct preparation of this substance from methyl dihydrobetulonate and comparison of it, the derived 2:4 - dinitrophenyl hydrazone, and the anhydride 8 obtained by selenium dioxide oxidation showed them to be identical in every respect. Therefore, they proposed four possible structures 9 , 10 , 11 and 12 for ceanothic acid.



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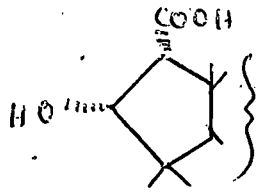

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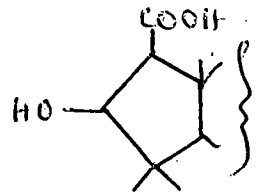

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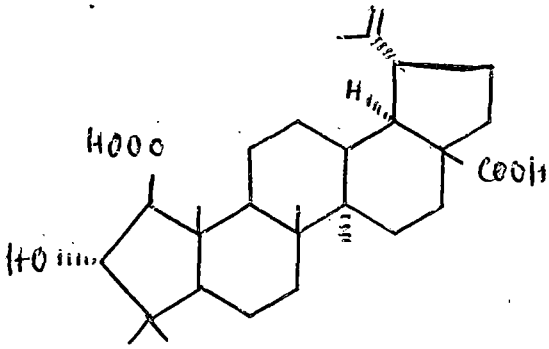

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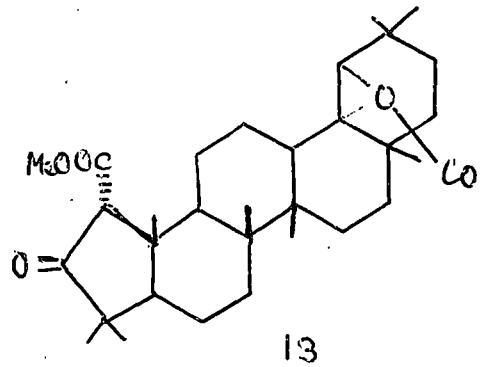

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They noted that, in dimethyl ceanothate, the methine hydrogens adjacent to the carbomethoxyl and hydroxyl groups were singlets, that is, although the hydrogens were on contiguous carbon atoms, the coupling constant was close to zero. In contrast, the ketone 13, derived from methyl ceanothate lactone, on reduction with sodium borohydride gave an epimeric lactone, called as methyl isoceanothate lactone. This substance showed, in its NMR spectrum, a doublet at τ 7·0, the methine hydrogens being coupled, and a quartet at τ 5·9[7], the hydrogen on the carbon

bearing oxygen being split by the adjacent methine and by the hydroxyl hydrogen. This suggested that in dimethyl ceanothate the hydrogen atoms were at an angle of about 90^{o} [13,14] and, therefore, in the trans relationship.

Further evidence supporting this view was obtained from a study of infrared spectra. Treatment of methyl dehydroceanothate lactone 13, with sodium methoxide resulted in a rapid epimerisation of the carbomethoxyl group - which must, therefore, be in the unstable configuration - and the formation of the isomeric methyl dehydroepiceanothate lactone. Reduction of this with sodium borohydride then gave methyl epiceanothate lactone. The results recorded by de Mayo and Starratt are given below (Table - 1).

Table-I

Compound	$\nu_{ester}(cm^{-1})$ *	$\nu_{hydroxyl}(cm^{-1})$ *	Concentration (%)†
Dimethyl dihydroceanothate	1733	3634 (sharp)	2·5
		~3510 (broad)	
		3634 (sharp)	1·2
		3640 (sharp)	0·2
Methyl isoceanothate lactone	1718	3582 (sharp)	0·7
		3580 (sharp)	0·1
Methyl epiceanothate lactone	1717	3682 (sharp), 3508 (broad)	1·9 ($CHCl_3$)‡
		3634 (sharp), 3508 (broad)	0·3

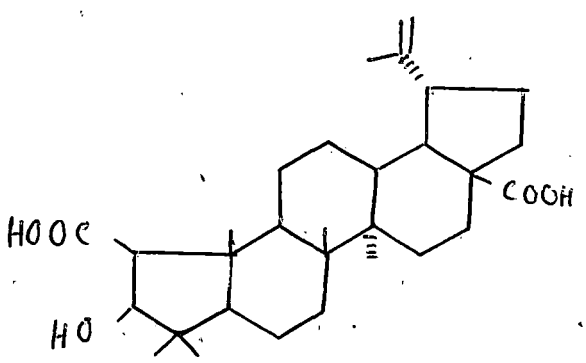
* Spectra taken in CCl_4 (Fisher spectranalyzed) unless specified otherwise

† Maximum concentration limited by compound solubility.

‡ Chloroform washed and dried to remove alcohol.

Methyl dihydroceanothate showed a normal unbonded ester and, in agreement, the hydroxyl group showed intermolecular bonding only in the most concentrated solution. In contrast, the epimeric methyl isoceanothate lactone showed a bonded ester group and a bonded hydroxyl band even in dilute solution. These results supported the trans and cis configurations, respectively, and they assumed that methyl epiceanothate was, also, cis.

Therefore, they proposed structures 9 and 12 for ceanothic acid. Inspection of models indicated that the β-carbomethoxyl group was under more severe non-bonded interaction than the α-epimer. In view of the ready epimerisation of methyl dehydro ceanothate lactone, they pfeferred the stereostructure 12 for ceanothic acid. In 1961, just prior to the publication of this paper, Mechoulam[15] published a paper in which he assigned the β-configuration to the carboxyl group in ring A and a cis relationship to this carboxyl and the adjacent hydroxyl group 14. The reasons which led him to propose this stereochemical assignment were based on the following grounds.



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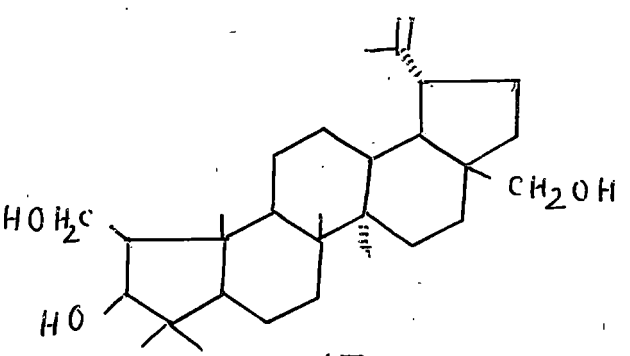

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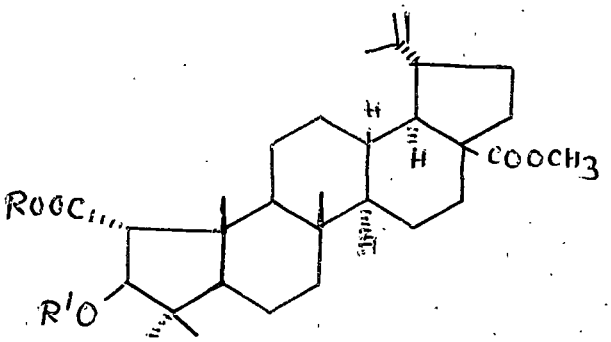
The hindrance of the secondary as well as tertiary - $COOCH_3$ group was shown by the fact that dimethyl ceanothate did not undergo basic hydrolysis with 10% potassium hydroxide solution. Examination of the model showed that a 1β - carboxyl group was considerably hindered but little hindrance was expected from 1α - configuration. This study led them to propose β - carboxyl configuration for the natural product. Again lithium aluminium hydride reduction of dimethyl 3-oxo-ceanothate gave a triol[2] 15, m.p. 226-28°, $(\alpha)_D$ 46° in EtOH, which was shown to be identical with a triol obtained by lithium aluminium hydride reduction of dimethyl ceanothate. They argued that, since for steric reasons, the hindered 3-oxo group in dimethyl - 3-oxo-ceanothate would be expected to give the 3β - ol by this type of reduction the natural product was in β-OH grouping. They further reported that this assignment was supported by a broad hydrogen - bonded hydroxyl IR absorption in the neighbourhood of 3500 cm^{-1} of a very dilute solution of triol 15 . Later Eade, Kornis and Simes[16,17] and Mechoulam[18] himself negated the idea of β - carboxyl group and cis relationship of the carboxyl and hydroxyl group with the supporting evidence in favour of the structure proposed by de Mayo and Starratt[5].

Eade et al[16] found that with lithium fluoride optics, a 0·05 M solution (CCl_4, 1 mm cell) of dimethyl ceanothate

showed strong absorption at 3629 cm^{-1} due to free hydroxyl group and medium absorption at 3513 cm^{-1} due to bonded hydroxyl group whereas a 0·01 M solution (CCl_4, 5 mm cell) had the same strong absorption at 3629 cm^{-1} but showed no absorption at 3513 cm^{-1}. They concluded that these results are consistent with the presence of intermolecular hydrogen bonding and absence of intramolecular hydrogen bonding. A study of molecular models indicated that intramolecular hydrogen bonding would be expected to occur where the methoxycarbonyl and hydroxyl group were cis[19].

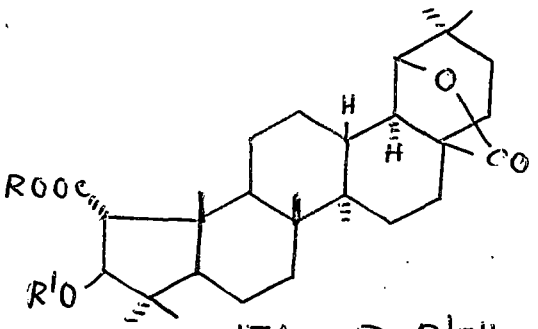
They also reported that lithium aluminium hydride reduction of dimethyl dihydroceanothate and the corresponding ketone dimethyl -3-oxo- dihydroceanothate gave different triols (The dimethyl -3-oxo-dihydro ceanothate gave a mixture, but only one product was isolated). This result was in contrast to that obtained by Mechoulam[15] who isolated the same triol on lithium aluminium hydride reduction of dimethyl ceanothate and the corresponding ketone dimethyl - 3-oxo- ceanothate. Eade, in his paper[16], refuted this conclusion drawn from this reduction on the basis of two possible factors ; (i) the reducing agent could chelate with hindering group and this might affect the stereochemical course of the reduction of the ketone, and (ii) epimerisation of the methoxycarbonyl group might occur during reduction. In 1958, Boyer et al[2] reported

that dimethyl ceanothate was stable to boiling 10% ethanolic potassium ethoxide for 2-hours. But Eade et al[16] found that with 20% methanolic potassium hydroxide and heating under reflux for 48 hours dimethyl ceanothate could be converted into a mixture containing very little or no starting material. The major product isolated in 50% yield was a monomethyl ester 16A. Methylation of 16A gave dimethyl ester 16B which was isomeric with dimethyl ceanothate. The corresponding acid isomeric with ceanothic acid was named isoceanothic acid. Lactonisation of compound 16A (H_2SO_4 / AcOH / Benzene) gave the lactone 17B indicating that it was the methoxycarbonyl group which had been hydrolysed by alkali. Both lactone 18A (normal series; prepared by lactonisation of dimethyl ceanothate) and lactone 17A (iso-series) was converted into the corresponding oxo-esters having different physical constants; lactone 18B (normal series; prepared by lactonisation of ceanothic acid) and 17A (iso-series) yielded the same nor-ketone 19



16A , $R = R^1 = H$

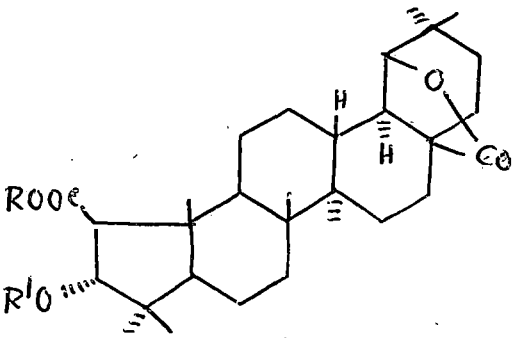
16B , $R = Me, R^1 = H$



17A , $R = R^1 = H$

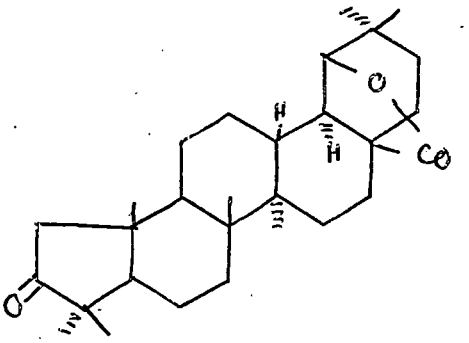
17B , $R = H, R^1 = COMe$

17C , $R = Me, R^1 = COMe$



18A , R = Me, R' = CO Me

18B , R = H , R' = CO Me



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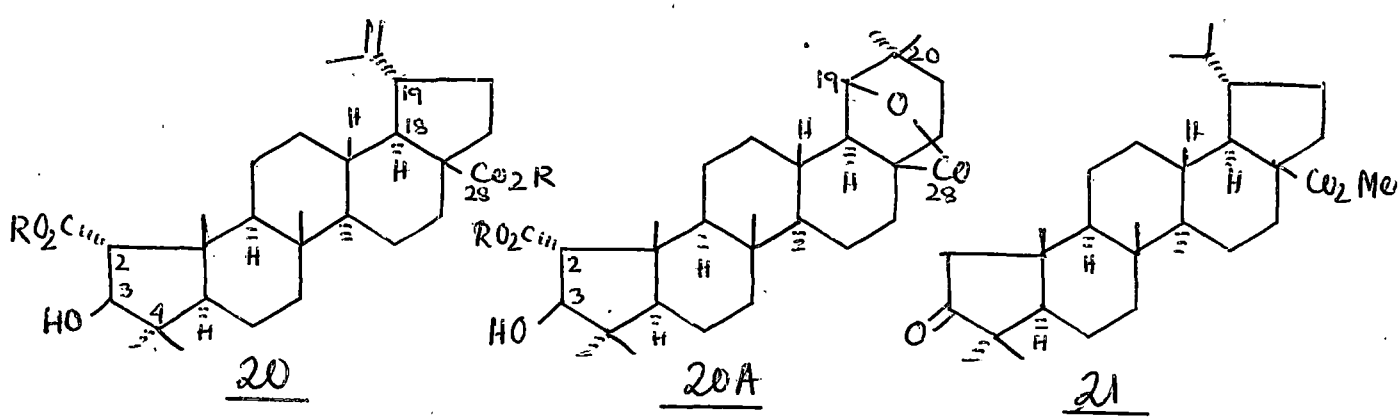
Thus the methoxycarbonyl group in ceanothic acid and that in isoceanothic acid were epimeric. These authors reported, from the examination of models, that if the methoxycarbonyl group in ring A had the β - configuration it would be under considerable non-bonded interaction. Such steric strain would be virtually absent if it possessed the α - configuration. Thus, they stated that the carboxyl group in ring A of ceanothic acid, probably possessed the more strained β - configuration leading to the α - assignment for the hydroxyl group. The ring A constituents of isoceanothic acid had been assigned a trans relationship because the infrared spectra of the

dimethyl ester 16B showed evidence of intermolecular hydrogen bonding. Consequently, formation of isoceanothic acid required opening of ring A in a reverse aldol type reaction to an A - seco derivative with subsequent reclosure.

Crowley[20], in 1962, reported the natural occurrence of a 2,3 - seco triterpene and this supported the suggested biogenesis[3] of ceanothic acid by ring closure of a similar 2,3 - seco derivative derived from 2 - hydroxy betulinic acid; the latter has recently been isolated along with ceanothic acid from Alphitonia whitei Braid[3]. Eade et al[16] found that the β - keto acid derived from the iso series either from 16 or 17 was quite stable. Oxidation of 16A or 17A gave the corresponding β -keto acid which could be dried in vacuo at 100° without decomposition. Conversion of the β - keto acid derived from 17A into the corresponding nor-ketone 19 was effected by heating in vacuo at 220°C. This stability, according to them, was due to difficulty in finding a cyclic planar transition state. In this connection, they cited the example of camphor-3-carboxylic acid which, according to them, is stereochemically similar and a stable β - keto acid. They could not isolate the β -keto acid derived from the normal series, that is, carboxyl with β - configuration which, they explained, was due to its ready decarboxylation.

Section C : Stereochemistry of Ceanothic Acid :

Final convincing proof for the stereochemistry of ceanothic acid was published in 1971 by Eade et al[21]. These authors[17], in 1964, observed that the ester group at C - 2 in dimethyl ceanothate 20 (R = Me) could be epimerised only with great difficulty, whereas in the corresponding ester, dimethyl ceanothate lactone 20A (R = Me) with a modified ring E area, it could be comparatively easily epimerised and was accompanied by simultaneous hydrolysis. They suggested[17] that this considerable difference in reactivity might be caused by a long-range conformational transmission effect. In 1971, Eade et al published a paper[21] in which they stated that a comparison of the coupling constants of the C - 2 and C - 3 protons of the four possible C 2 - CO_2Me / C 3 -OH isomers and their acetates in the dimethyl ceanothate series (carbon skeleton type 20) with those of the corresponding compounds in the lactone series (carbon skeleton type 20 A) would reveal any significant differences in the shape of ring A between the relevant compounds.



20 20A 21

They also expected that explanation might be found of the signal reported[5] for the C - 3 proton in the NMR spectrum of dimethyl ceanothate, viz. a singlet, which was not in agreement with the structure then assigned to ceanothic acid even if ring A existed in the α - envelope conformation[22,23]. They further expected that the preparation of the complete group of four isomers in each series would resolve the controversy whether the "methyl epiceanothate" of de Mayo and Starratt has a cis or trans relationship of the ring A substituents[5,17].

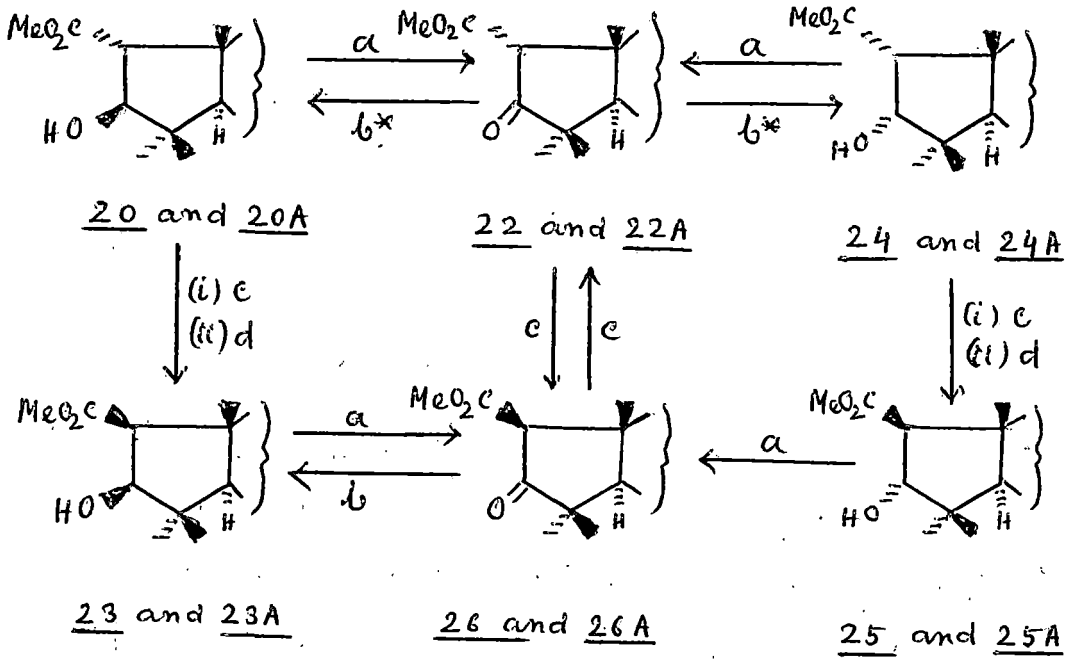
Eade et al[24] from some chemical and physical studies, described below, finally proposed[24] structure 20 (R = H) for ceanothic acid in which the ring A substituents, while still having a trans relationship, possess the configurations opposite to those originally put forward by de Mayo and Starratt[5].

Preparation of the Dimethyl Ceanothate Series :

Of the four possible isomers in the dimethyl ceanothate series, two have been previously prepared. One is dimethyl ceanothate itself 20 (R = Me) and the other is its C - 2 epimer 23 that is , methyl 3β - hydroxy - 2β -methoxycarbonyl-A(1)-norlup-20(29)-en-28-oate (Scheme 1) reported by Eade, Kornis and Simes[16] who incorrectly formulated it as the trans isomer

of dimethyl ceanothate epimerised at both C - 2 and C - 3. This isomer was originally given the name dimethyl epiceanothate in accordance with the nomenclature used by de Mayo and Starratt. This isomer 23 was first prepared by epimerisation of the C - 2 methoxycarbonyl group by prolonged treatment of dimethyl ceanothate with concentrated methanolic sodium methoxide to give a mixture from which the half ester of 23, methyl 2β - carboxy - 3β - hydroxy - A(1) - norlup-20(29)-en-28-oate could be isolated after a lengthy purification procedure; methylation with diazomethane then gave 23. Eade et al[21] prepared this compound according to the procedure used by de Mayo and Starratt[5] for the analogous compound in lactone series. Thus oxidation of dimethyl ceanothate with Jones reagent gave the known methyl 2α - methoxycarbonyl-3-oxo-A(1)-norlup-20(29)-en-28-oate, that is, dimethyl dehydroceanothate 22 which was rapidly epimerised by alkali to an equilibrium mixture containing 40% of starting material and 60% of the isomer, epimeric at C - 2, methyl 2β - methoxycarbonyl-3-oxo-A(1)-norlup-20(29)-en-28-oate 26, that is, dimethyl epidehydroceanothate.

Scheme - 1



a, Jones reagent; b, Sodium borohydride; c, Sodium methoxide; d, Diazomethane. * 22 → 20 + 24 but 22A → 24A only

Eade et al, in 1964 described[17] the corresponding half-ester, methyl 2β- carboxy -3-oxo-A(1)-norlup-20(29)-en-28-oate, that is, monomethyl epidehydroceanothate. Reduction of 26 with sodium borohydride gave the cis isomer 23 as sole product; on oxidation with Jones reagent it gave back the parent ketone 26 quantitatively.

Reduction of dimethyl dehydroceanothate 23 with sodium borohydride gave a 1 : 1 mixture of dimethyl ceanothate

20 (R = Me) and its C - 3 epimer 24 which were readily separated by column chromatography. Oxidation of 24 with Jones reagent gave back the original ketone 22 quantitatively.

The fourth isomer, methyl 3α - hydroxy- 2β -methoxycarbonyl-A(1)-norlup-20(29)-en-28-oate 25, was prepared by long heating of a solution of methyl 3α - hydroxy-2α -methoxycarbonyl-A(1)-norlup-20(29)-en-28-oate 24 in methanolic sodium methoxide whereupon partial epimerisation of the C 2 - methoxycarbonyl group occured. After remethylation of the total crude product, they separated the isomer 25 from starting material by column chromatography; the identity of 25 was confirmed by its oxidation with Jones reagent to the ketone 26.

Eade et al have also reported the conversion of the above alcohols into their corresponding acetates.

Preparation of the Lactone Series :

Three of the four isomers in this series have previously been reported[5,16]. Methyl ceanothate lactone 20 A (R = Me) has been prepared from ceanothic acid by formic acid catalysed lactonisation followed by hydrolysis and methylation[5] and also by lactonisation of dimethyl ceanothate in acetic acid and sulphuric acid followed by deacetylation[17].

The compounds 23 A and 24 A have been prepared by borohydride reduction of the corresponding ketones 22 A and 26 A. Eade et al also prepared the above three isomers using the same procedure described above. The fourth and previously unknown isomer, 3α-hydroxy-2β-methoxycarbonyl-A(1)-nor-18α-olean-28 $\rightarrow$ 19β-olide 25 A, was prepared by isomerisation of 3α- hydroxy - 2α- methoxycarbonyl-A(1)-nor-18α-olean-28 $\rightarrow$ 19β-olide 24 A with sodium methoxide in methanol, followed by remethylation with diazomethane and purification by chromatography on alumina. Its identity was confirmed by oxidation to the ketone 26 A.

The acetates of all four isomeric alcohols in the lactone series were also prepared by Eade et al.

These authors also reported that the epimerisations of both the ketones in each series exist in reversible equilibrium; that is, if either 22 or 26 is treated with sodium methoxide in methanol a mixture of 60% 26 and 40% 22 results and a similar mixture of 26 A and 22 A is obtained from either 22 A or 26 A.

N. M. R. Results :

Eade et al[21] gave a list of coupling constants between the protons on C - 2 ~~and C - 2~~ and C - 3 for each isomer and its

acetate in both series and also a list of the vicinal coupling constants ($J_{2,3}$) of the four isomers calculated for the three possible ring A conformations from the dihedral angles measured from Dreiding models. Of these three possible conformations, the α- envelope seemed least likely since examination of the models showed strong 10β- methyl / 4β- methyl interaction which would be expected to destabilise this conformation; in addition, Fishman[25] in his investigation on analogous 16,17-disubstituted steroids lacking these methyl / methyl interaction did not consider the α- envelope. The only significant difference between the three conformations were the two trans couplings in the α- envelopes[25] and therefore these results, although excluding the α- envelope, did not distinguish between the other two conformations. The observed couplings taken by Eade et al[21] were clearly consistent with their proposed formula for ceanothic acid 20 (R = H) and do not conform with the original stereochemistry assigned by de Mayo and Starratt[5].

Table - II shows that there are no significant differences between the coupling constants of the C - 2 and C - 3 protons in members of dimethyl ceanothate series compared with those of the analogous compounds in the lactone series and hence this investigation did not yield any evidence about the long range conformational transmission effect which had been proposed[17] to explain differences in reactivity in the

ring A area between the two series. The coupling constants in Table - II, together with interconversions summarized in scheme - 1, established unequivocally that in the epiceanothate series the ring A substituents are cis to one another, each having the β- configuration.

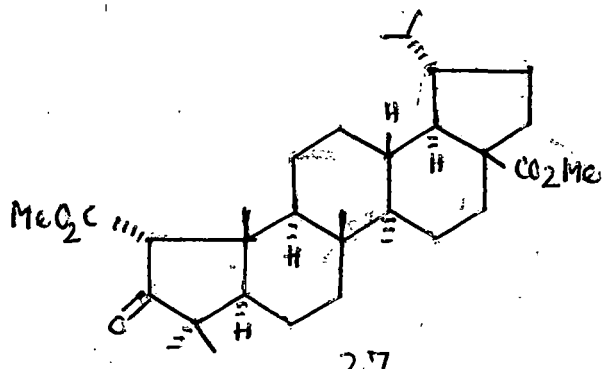
Table - II

Coupling constants of C - 2 and C - 3 protons

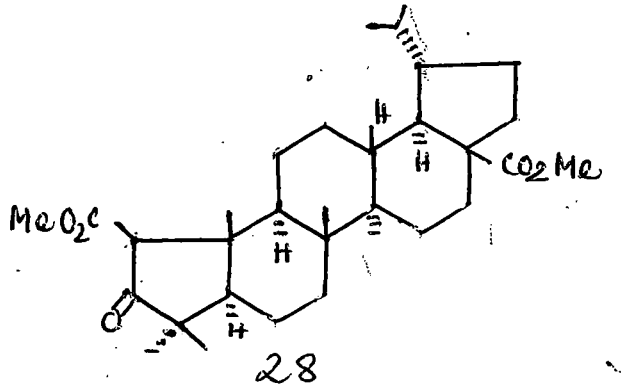
	Configurations of Ring A Substituents			
	$2\alpha, 3\beta$	$2\beta, 3\beta$	$2\alpha, 3\alpha$	$2\beta, 3\alpha$
Observed $J_{2,3}$ (Hz)				
Dimethyl ceanothate series :				
C 3 - OH	1·0	7·3	7·0	9·0
C 3 - OAc	0·2	7·6	7·6	9·5
Lactone series :				
C 3 - OH	1·0	7·4	7·0	9·0
C 3 - OAc	0	7·6	7·7	9·5
Calculated $J_{2,3}$ (Hz)				
α - Envelope	1·8-2·9	8·2	8·2	1·8 - 2·9
β - Envelope	-0·3-0·0	6·2-6·9	5·7-6·7	5·9 - 7·1
Half-chair	0·3-0·8	7·8-8·0	7·5-7·9	3·5 - 5·0

In addition to the evidence presented above in relation to the vicinal coupling between the protons on C - 2 and C - 3, the long-range (4σ) coupling between the 10β- methyl group and the C - 2 proton provides further support for their assignment. This coupling was studied using the following three pairs of isomers ; (i) methyl dehydroceanothate lactone 22 A and its 2β- epimer 26 A ; (ii) dimethyl dehydroceanothate 22 and its 2β- epimer 26 ; (iii) dimethyl dihydrodehydroceanothate, that is, methyl 2α- methoxycarbonyl - 3-oxo-A(1)-norlupan-28-oate 27 and its 2β-epimer 28. The last pair of isomers were prepared from dimethyl dihydroceanothate by methods identical with those used to prepare 22 and 26 from dimethyl ceanothate. In each of these six compounds the C - 2 proton appeared as a singlet at approximately δ 3·0 ; and, this signal, they observed for each of the 2β- methoxycarbonyl (epi) derivatives was broad ($W_{\frac{1}{2}}$ 2·5 Hz) compared with that in each of the 2α- methoxycarbonyl compounds ($W_{\frac{1}{2}}$ 1·5 Hz). This broadening was confirmed by spin-spin decoupling; decoupling of the C - 2 proton signal at δ 3·08 in dimethyl epidehydroceanothate 26 increased the intensity of the C - 10 methyl signal at δ 1·06 markedly, while irradiation of this methyl frequency resulted in a sharpening of the C - 2 signal ($W_{\frac{1}{2}}$ became 1·6). They also observed similar sharpening of the C - 2 proton signal from $W_{\frac{1}{2}}$ 2·5 Hz to $W_{\frac{1}{2}}$ 1·6 Hz when the relevant methyl signal in

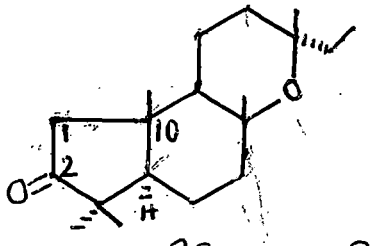
both 26 A and 28 was irradiated. Similar long range (46) coupling between the 1α-proton and the 10β - methyl was observed in the nor-diterpene colensan - 2-one 29[26]. The observed couplings, they stated, was due to the pseudo-axial character of the 2α - hydrogens in 26, 26 A, and 28 and consequently supported the half-chair or β - envelope conformation of ring A for these derivatives when the 2α - proton and 10β - methyl possessed some degree of co-planarity.



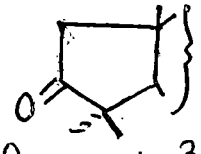
27



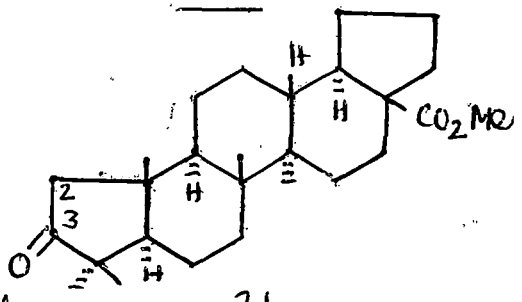
28



29



30 and 30A



31

C. D. Results : The c. d. of the two epimeric ketones 22 and 26 together with that of the corresponding norketone 30 and also the corresponding sets of three ketones in the lactone series 22A, 26A and 30A and in the dimethyl dihydroceanothate series 27, 28 and 31 have been studied. But the changes in c. d. between members of each set did not yield any information about the configuration of the C-2 methoxycarbonyl group.