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TRITERPENOIDS—XXXVII

THE STRUCTURE AND STEREOCHEMISTRY OF LANTANOLIC ACID—A NEW TRITERPENOID FROM *LANTANA CAMARA*

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Abstract—The structure and stereochemistry of Lantanolic acid—a new triterpene from *Lantana camara* has been established as Ia by chemical and physical methods.

THE plant *Lantana camara*, which during flowering and fruiting stages causes severe photosensitization and icterus[1] in sheep, was first chemically examined by Louw[2] who isolated two triterpenes called lantadene A and lantadene B from the leaves. The former was to be causative factor for photosensitization and icterus, whereas the latter was found to be physiologically inactive. Later, the problem was reinvestigated by Barton *et al.*[3,4] who characterized lantadene A as rehmannic acid and also elucidated the structure of lantadene B as 22β (β,β-dimethylacryloyloxy)-3-oxo-olean-12-en-28-oic acid. The isolation of two new triterpenes called lantanolic acid and lantic acid from the leaves of *L. camara* was reported by us in a preliminary communication.[5] We now wish to present evidence leading to the structure Ia for lantanolic acid.

Lantanolic acid (Ia), $C_{30}H_{46}O_4$, M^+ 470, m.p. 306–309° (dec), $[\alpha]_D^{38°}$ + 151° ($CHCl_3$), formed a monomethyl ester (Ib), $C_{31}H_{48}O_4$, M^+ 484, m.p. 197–198°, $[\alpha]_d^{33°}$ + 156° ($CHCl_3$). Methyl lantanolate (Ib) showed pink → violet colouration in the Liebermann–Burchard test and a pale yellow colour with tetranitromethane. The UV spectrum of methyl lantanolate showed maximum at 204 mμ (log ε 3·8) indicating the presence of a trisubstituted double bond. The IR spectrum (KBr) of methyl lantanolate exhibited bands at 3575 cm^{-1} (OH), and 1715 cm^{-1} (—$COOCH_3$). Methyl lantanolate gave an mono-oxime, which on treatment with acetic anhydride and fused sodium acetate furnished an oxime diacetate, [IR spectrum (Nujol): 1755 and 1205 cm^{-1} (=N—OAc), 1730 and 1235 cm^{-1} (OAc), 1715 cm^{-1} (—$COOCH_3$) and 1625 cm^{-1} ($\rangle C{=}N-$)] and not a nitrile, thereby establishing the presence of a potential CO function in lantanolic acid as a keto group. Methyl lantanolate on refluxing with methanol and conc sulphuric acid furnished a ketal (Ic), which gave back methyl lantanolate on treatment with dil hydrochloric acid in

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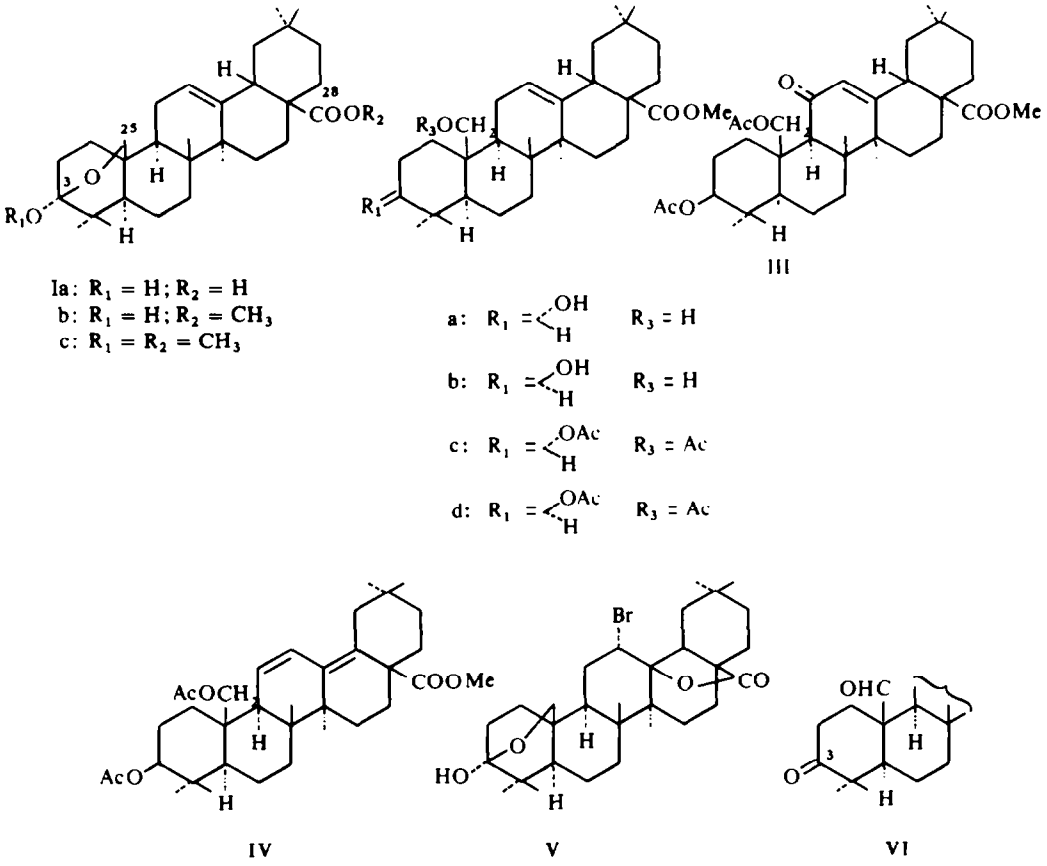
tetrahydrofuran. The IR spectrum of the ketal did not show any band for OH group. The keto group in lantanolic acid is therefore present as a hemiketal system—a conclusion which would be further evident from the following discussion.

On reduction with potassium borohydride, methyl lantanolate furnished two isomeric diols: diol A (IIa), and diol B (IIb). Diol B, the major product (90%), appeared to be the equatorial isomer as it moved slower than diol A on TLC.[6] Both the diols A and B formed diacetates: diol A diacetate (IIc) and diol B diacetate (IId). The NMR spectra of diol B and its diacetate clearly indicated the presence of a secondary as well as a primary OH group in diol B. The secondary OH group was obviously formed by the reduction of the potential keto group in methyl lantanolate. On oxidation with CrO_3 in glacial acetic acid under reflux diol B diacetate yielded an α,β-unsaturated ketone (III), λ_{max} 248 mμ (log ε 4·1). Diol B diacetate on oxidation with selenium dioxide in glacial acetic acid furnished a diene (IV), which showed triple UV absorption maxima at 243, 249 and 260 mμ, typical of $\Delta^{11:12, 13:18}$-dienes of the oleanane series.[7] Lantanolic acid should therefore be a triterpene of the oleanane series having a 12:13-double bond. Under comparable conditions, the rate of saponification of methyl lantanolate was found to be the same as that of methyl oleanolate,[8–10] thus suggesting the probable location of the carboxyl group in lantanolic acid at C-17 and this was further confirmed by the formation of a monobromo γ-lactone (V), $C_{30}H_{45}O_4Br$, M^+ 548 (Br^{79}) and 550 (Br^{81}), by treatment of lantanolic acid with bromine in acetic acid.[11] Its IR spectrum (Nujol) showed only a single CO band at 1755 cm^{-1} (γ-lactone) thus supporting our previous conclusion that the keto group in lantanolic acid is not free but forms part of a hemiketal system.

Diol B (IIb) on oxidation with pyridine–CrO_3 complex furnished a keto-aldehyde (VI) [IR spectrum (Nujol): broad band at 1700–1730 cm^{-1} (CO); 2740 cm^{-1} (—CHO)], which responded to Zimmermann's colour test for 3-keto group[8] thereby suggesting the presence of C-3 OH group in diol B, which was obviously formed by the borohydride reduction of the potential keto group at C-3 in methyl lantanolate. Methyl lantanolate itself, however, did not respond to Zimmermann's colour test for 3-keto group. If the potential keto group in methyl lantanolate is located at C-3 position, the potential primary OH group must be fixed at C-25 as other two probable positions namely C-23 and C-24 were ruled out because both methyl hederagonate, i.e., methyl 23-hydroxy-3-oxo-olean-12-en-28-oate (having a keto group at C-3 and a primary OH group at C-23)[8] and icterogenin, i.e., 22β-angeloyloxy-24-hydroxy-3-oxo-olean-12-en-28-oic acid (having a keto group at C-3 and a primary OH group at C-24),[8] unlike methyl lantanolate, undergo retroaldolization in presence of alkali and also give positive Zimmermann's colour reaction for 3-keto-group. It appeared therefore, that the hemiketal linkage in lantanolic acid involves the keto-group at C-3 and the primary OH group at C-25.

Correlation of lantanolic acid with oleanolic acid was achieved in the following manner. Wolff–Kishner reduction of methyl lantanolate (Ib) followed by treatment with diazomethane furnished a product (VIIa), which on oxidation with pyridine–CrO_3 complex furnished the aldehyde (VIIb) [IR spectrum ($CHCl_3$): 2690, 2770 cm^{-1} (—CHO)]. This aldehyde on Wolff–Kishner reduction under anhydrous conditions,[12] followed by treatment with diazomethane, yielded a product which was identified (m.p., mixed m.p. and IR spectra) as 3-deoxy methyl oleanolate (VIII). Thus lantanolic acid was proved to be a pentacyclic triterpene of the oleanane



SCHEME 1

series having a 12:13-double bond and a carboxyl group at C-17 position. As the hemiketal system was previously shown to involve C-3 keto group and the primary OH group at C-25, lantanolic acid may be represented as Ia.

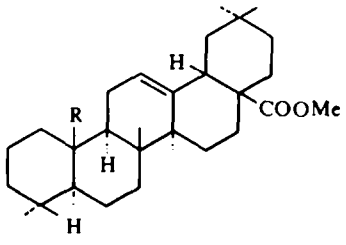
Supporting evidence for the proposed structure Ia for lantanolic acid was adduced from the NMR and mass spectral studies of methyl lantanolate and its derivatives.

The NMR spectrum (60 Mc. in $CDCl_3$) of methyl lantanolate (Ib) showed five sharp singlets at 0·67 (3H), 0·90 (6H), 0·98 (3H), 1·02 (3H) and 1·13 δ (3H) corresponding to the six tertiary Me groups, a singlet at 2·58 δ (1H, OH; disappeared on exchange with D_2O), a sharp singlet at 3·6 δ (3H, —COOC$\underline{H}_3$),[13] a triplet at 5·35 δ (1H, C-12 vinyl proton)[13] and a pair of doublets centred at 4·27 and 3·85 δ (J = 9 c/s) attributed to the two nonequivalent methylene protons of the hemiketal ring system.[14] The bands at 4·27 and 3·85 δ are further split probably due to long range coupling (J = 2·5 and 1 c/s respectively) with one of the C-1 protons. In the NMR spectrum of the diol B the two protons of the primary hydroxymethylene group (—C$\underline{H}_2$OH) at C-10 appeared as a sharp singlet at 4·03 and the C-3 axial proton[15] as a triplet (J = 7·5 c/s) centred at 3·27 δ. In the NMR spectrum of diol B diacetate, the former

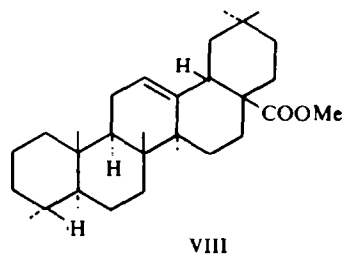
was shifted to 4·45 δ (singlet, 2H, $-\underline{CH_2}-O-CO-CH_3$) and the latter to 4·52 δ (t, J = 7·5 c/s; 1H; $>\underline{CH}-O-Ac$) as expected.

The mass spectrum (obtained with an AEI MS9 mass spectrometer) of methyl lantanolate showed principal fragmentation peaks as presented in scheme I. The peak at *m/e* 262 (ion *a*) resulting from the retro Diels–Alder fragmentation[16] indicated the absence of any other substitution in rings C, D and E of lantanolic acid except the carboxyl group. An intense peak at *m/e* 241 may be explained as due to the ion *b*. Its composition was confirmed by high resolution mass measurement. The mass spectra of the diol B (IIb) and diol B diacetate (IId) showed peaks at *m/e* 455 and 437 respectively.

Incidentally, it may be mentioned that lantanolic acid is the first example of a triterpene having an oxygen function at C-25.

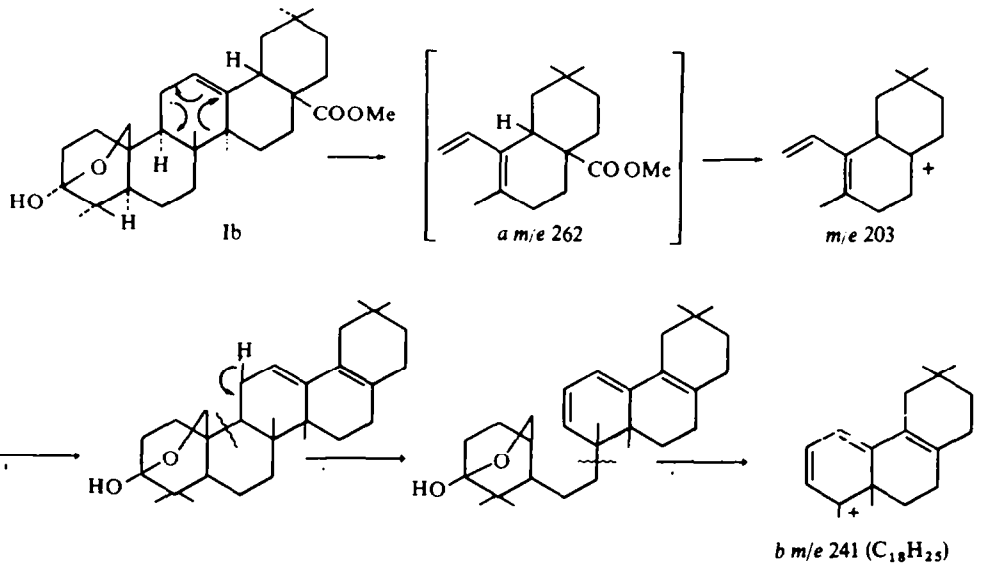


VIIa: R = CH_2OH
b: R = CHO



VIII

SCHEME I



EXPERIMENTAL

M.ps (uncorrected and recorded in a bisulphate bath). Pet. ether (b.p. 60–80). Brockmann's alumina (S. Merck) and silica gel (supplied by National Chemical Laboratory, Poona, India) were used for column

chromatography. Acid-washed alumina refers to Brockmann's alumina deactivated with 5% of 10% AcOH. Optical rotations (in $CHCl_3$ soln unless otherwise specified). Mass spectra (an A.E.I. MS 9 mass spectrometer operating at 70 eV).

Isolation of lantanolic acid (Ia). Air-dried powdered leaves of *L. camara* (1 kg) was Soxhleted with light petroleum. The solvent was removed by distillation and the residue was taken up in ether. The ethereal soln was washed with 5% KOH aq in the usual way to separate the acidic and neutral fractions. The crude acid fraction thus obtained was chromatographed on a column of Brockmann's alumina and the fraction eluted with $CHCl_3$–MeOH (1:1) was crystallized from $CHCl_3$–EtOH (95%) to yield lantanolic acid (120 mg), m.p. 306–309° (dec), $[\alpha]_D^{38°}$ +151°. (Found: C, 76·38; H, 9·92; M^+ 470. $C_{30}H_{46}O_4$ requires: C, 76·59; H, 9·78%; Mol. wt. 470).

Methyl lantanolate (Ib). Lantanolic acid (100 mg) was esterified with ethereal diazomethane. Working up in the usual way and crystallization from light petroleum and also from MeOH yielded Ib (70 mg), m.p. 197–198°, $[\alpha]_D^{33°}$ +156°. (Found: C, 76·51; H, 9·61; M^+ 484. $C_{31}H_{48}O_4$ requires: C, 76·85; H, 9·91%; Mol. wt. 484).

Methyl lantanolate (421 mg) was refluxed with 20% ethanolic KOH (25 ml) for 3 hr on steam bath. The products were separated into acid (21 mg) and neutral parts (375 mg). The acid part (lantanolic acid) was crystallized from $CHCl_3$–EtOH (95%), m.p. 306–309°.

Mono-oxime of methyl lantanolate. Methyl lantanate (100 mg) and hydroxylamine hydroxcloride (150 mg) in pyridine soln (3 ml) was heated on a steam bath for 2 hr. The mixture was worked up in the usual way and crystallized from aqueous EtOH, m.p. 222–224°. (Found: C, 75·19; H, 10·15; N, 3·35. $C_{31}H_{49}O_4N$ requires: C, 74·55; H, 9·82; N, 2·81%).

The diacetate of the above oxime was prepared by refluxing the oxime (300 mg) with Ac_2O (15 ml) and fused NaOAc (700 mg) for 4 hr. The crude product which was worked up in the usual way was crystallized from light petroleum (b.p. 40–60°), m.p. 187–188°. (Found: C, 72·03; H, 8·61; M^+ 583. $C_{35}H_{53}O_6N$ requires: C, 72·04; H, 9·09%; Mol. wt. 583).

KBH_4 *Reduction of methyl lantanolate.* Methyl lantanolate (500 mg) in MeOH (25 ml) was kept with KBH_4 (400 mg) at room temp for 48 hr and the product was worked up in the usual way. The crude mixture was chromatographed over acid-washed alumina. Elution of the column with light petroleum–benzene (2:1) yielded a glassy residue which was crystallized from benzene–light petroleum to yield IIa (38 mg), $C_{31}H_{50}O_4$, m.p. 226–230°. Further elution of the column with light petroleum–benzene (1:1) yielded a solid fraction, crystallized from aqueous MeOH to yield IIb (425 mg), $C_{31}H_{50}O_4$, m.p. 232–234°, $[\alpha]_D^{31°}$ +90°. (Found for IIa: C, 76·21; H, 10·12. Found for IIb: C, 76·32; H, 10·24; M^+ 486. $C_{31}H_{50}O_4$ requires: C, 76·54; H, 10·28%; Mol. wt. 486).

Acetylation of diol A (IIa). Diol A diacetate (IIc) was prepared by heating diol A (50 mg) with pyridine (1 ml) and Ac_2O (1 ml) on a steam bath for 6 hr. The product was crystallized from light petroleum, m.p. 179–180°, $[\alpha]_D^{31°}$ +56°. (Found: C, 73·43; H, 9·35. $C_{35}H_{54}O_6$ requires: C, 73·68; H, 9·47%).

Acetylation of diol B (IIb). Diol B diacetate (IId) was prepared by heating diol B (1·4 gm) with pyridine (10 ml) and Ac_2O (5 ml) on a steam bath for 6 hr. The product was crystallized from aqueous MeOH, m.p. 236–239°, $[\alpha]_D^{37°}$ +74° (Found: C, 73·51; H, 9·34; M^+ 570. $C_{35}H_{54}O_6$ requires: C, 73·68; H, 9·47%; Mol. wt. 570).

Oxidation of IId *to* III. A boiling soln of IId (500 mg) in glacial AcOH (10 ml) was treated with a soln of CrO_3 (550 mg) in AcOH (90%, 9 ml). After refluxing for 2 hr at room temp the mixture was diluted with water and worked up in the usual way. The product was purified by chromatography over silica gel and crystallized from $CHCl_3$–MeOH, m.p. 242–243°, $[\alpha]_D^{37°}$ +111°, (Found: C, 71·61; H, 8·67; M^+ 584. $C_{35}H_{52}O_7$ requires: C, 71·92; H, 8·90%; Mol. wt. 584).

SeO_2 *Oxidation of diol B diacetate* (IId). Diol B diacetate (80 mg) in glacial AcOH (9 ml) was refluxed for 14 hr after addition of SeO_2 (20 mg). The product was worked up in the usual way and crystallized from $CHCl_3$–MeOH, (IV), m.p. 250–270°. The product was found to be pure by TLC but further attempts to purify the compound was not made.

Oxidation of diol B (IIb). Diol B (300 mg) in pyridine (5 ml) was added to CrO_3–pyridine complex (prepared from 400 mg CrO_3 and 20 ml of pyridine) and kept overnight at room temp. After working up, the crude product was chromatographed over a column of silica gel and the fraction eluted with $CHCl_3$–benzene (2:1) mixture, was crystallized from $CHCl_3$–EtOH (95%), (VI), m.p. 225–227°. (Found: C, 77·39; H, 9·67. $C_{31}H_{46}O_4$ requires: C, 77·18; H, 9·54%).

Monobromo-γ-lactone (V) *of* Ia. A soln of lantanolic acid (Ia, 250 mg) and AcONa (1 g) in glacial AcOH (30 ml) was treated dropwise with 3% Br_2 in AcOH (10 ml). The mixture was shaken for a few min and

left for 5 hr at room temp after which it was poured onto water containing $Na_2S_2O_3$ when white ppt separated. It was filtered, washed with water and dried. The product was purified by chromatography over silica gel and the fraction eluted with $CHCl_3$–MeOH (19:1), was crystallized from $CHCl_3$–EtOH, (V), m.p. 251–252° (dec). (Found: C, 64·95; H, 8·50. $C_{30}H_{45}O_4Br$ requires: C, 65·57; H, 8·20%).

Ketal (Ic) *of methyl lantanolate* (Ib). Ib (100 mg) was dissolved in methanol (10 ml) and refluxed on a steam-bath for 2 hr with con. H_2SO_4 (3 drops). On concentration a crystalline material was obtained, filtered, washed with water, dried and then crystallized from $CHCl_3$–MeOH, m.p. 226–229°. (Found: C, 77·00, H, 10·20; M^+ 498. $C_{32}H_{50}O_4$ requires: C, 77·11; H, 10·04%; Mol. wt. 498).

The ketal (Ic, 40 mg) was dissolved in THF (10 ml) and refluxed on a steam-bath for 1 hr with dil HCl (5%, 3 ml). The product was filtered after dilution with water and crystallized from light petroleum and then recrystallized from aqueous MeOH, m.p. 197–198°, $[\alpha]_D^{30°}$ +155°. The product was identified as methyl lantanolate by mixed m.p. and IR spectra.

Wolff–Kishner reduction of methyl lantanolate (Ib). To a soln of methyl lantanolate (1 g) in a mixture of abs. EtOH (30 ml), diethylene glycol (30 ml) and dioxan (15 ml), hydrazine hydrate (85%, 30 ml) was added and refluxed for 1 hr. Solid KOH (8 g) was then added and enough solvent was distilled off to raise the boiling temp to 200–205° and refluxing was continued for 5 hr and then poured onto crushed ice, acidified, extracted with ether and separated into acid and neutral parts in the usual way. The acid part was esterified with diazomethane in the usual way. The crude ester was chromatographed over a column of Brockmann's alumina and the fraction eluted with benzene was crystallized from $CHCl_3$–MeOH, (VIIa), m.p. 172–173°, $[\alpha]_D^{35°}$ +98°. (Found: C, 79·38; H, 10·47; M^+ 470. $C_{31}H_{50}O_3$ requires: C, 79·15; H, 10·64%; Mol. wt. 470).

Sarett oxidation of VIIa. Compound VIIa (500 mg) was dissolved in dry pyridine (5 ml) and added to CrO_3–pyridine complex (600 mg CrO_3 and 20 ml dry pyridine) cooled in freezing mixture with stirring and kept overnight at room temp. The mixture was then worked up in the usual way and purified by column chromatography and crystallized from $CHCl_3$–EtOH, (VIIb), m.p. 216–220°, $[\alpha]_D^{37°}$ +110°. (Found: C, 79·81; H, 10·07. $C_{31}H_{48}O_3$ requires: C, 79·49; H, 10·26%).

Wolff–Kishner reduction of VIIb. The above VIIb (250 mg) was dissolved by boiling in freshly distilled diethylene glycol (25 ml) containing metallic Na (350 mg). Anhydrous hydrazine was then distilled in so that the mixture refluxed freely at 180–185° and the refluxing was continued for 10 hr. Some amount of hydrazine was then distilled off so that the mixture refluxed freely at 210–215° and refluxing was continued for further 12 hr. The mixture was then poured onto crushed ice, acidified with dil HCl and then worked up in the usual way. The crude product was esterified with ethereal diazomethane and purified by column chromatography to give VIII (50 mg), m.p. 169–170°, $[\alpha]_D^{30°}$ +74·3° (Py). (Found: C, 81·78; H, 11·13. $C_{31}H_{50}O_2$ requires: C, 81·94; H, 11·01%.

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