

Triterpenoids. Part XIX. The Constitution of Lantadene B.*

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Lantadene B has been shown to have the composition $C_{35}H_{52}O_5$. On alkaline hydrolysis it affords $\beta\beta$ -dimethylacrylic acid and 22β -hydroxyoleanonic acid. On the basis of this and other evidence lantadene B is shown to be 22β -($\beta\beta$ -dimethylacryloyloxy)oleanonic acid.

IN previous parts of this series (Barton and de Mayo, *J.*, 1954, 887, 900) the constitution of icterogenin, the photosensitivity-conferring constituent of *Lippia rehmanni* Pears, was elucidated as (I; R = angeloyloxy), and the occurrence of a further triterpenoid acid, rehmannic acid (II; R = angeloyloxy, R' = H), was demonstrated. Substances of obviously related chemical constitution have been isolated by Louw (*Onderstepoort J. Vet. Sci.*, 1943, 18, 197; 1948, 23, 233; 1949, 22, 321, 329) from *Lantana camara* L. and have been designated lantadene A and B. Lantadene A is stated to have the composition $C_{31}H_{44}O_5$ and to be physiologically active in the same way as icterogenin. Lantadene B on the other hand is regarded as having the composition $C_{33}H_{48}O_5$ and is said to be physiologically inactive. An investigation of the constitutions of the lantadenes was initiated some years ago by two of us (O. J. and G. W. P.) at Zürich. In view of the recently reported work (Barton and de Mayo, *loc. cit.*) on the *Lippia* constituents, and, in particular, having regard to the close resemblance (see below) of lantadene B and rehmannic acid, it was felt desirable to collaborate in the investigation of the chemistry of lantadene B. The current paper describes work on a lantadene mixture isolated at Zürich.

Chromatography of the crude lantadene over silica gel (cf. Barton and de Mayo, *loc. cit.*, p. 887) afforded only one pure substance in good yield. This was shown to be lantadene B by its physical constants, by direct (mixed m. p.) comparison with an authentic specimen of lantadene B very kindly provided by Dr. P. G. Louw, and by the identity of infra-red spectra in bromoform solution. Combustion data on lantadene B and its derivatives, and the degradational sequence outlined below, showed that its true molecular formula was $C_{35}H_{52}O_5$, the same as that established (Barton and de Mayo, *loc. cit.*, p. 900) for rehmannic acid. Lantadene B gave an oxime and a 2:4-dinitrophenylhydrazone showing, in agreement with Louw (*loc. cit.*), the presence of a carbonyl

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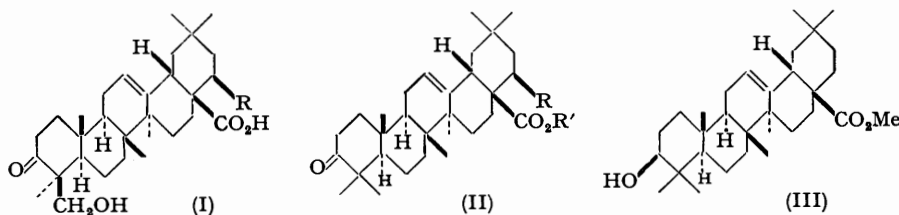
group. The physical constants recorded by us for lantadene B, its oxime, and 2:4-dinitrophenylhydrazone are almost identical with those which we reported for rehmmanic acid and its derivatives. Lantadene B and rehmmanic acid gave a slight melting-point depression on admixture, but the corresponding derivatives did not. The infra-red spectra of the 2:4-dinitrophenylhydrazones differed only at three frequencies (1035, 1070, and 1125 cm^{-1} ; chloroform solution) in the finger-print region. In the carbonyl region in the infra-red (see Table) rehmmanic acid, icterogenin, and lantadene B had identical spectra.

Nevertheless several pieces of experimental evidence contraindicated the identity of lantadene B and rehmmanic acid. Lantadene B was an acid affording a methyl ester, but this could not be obtained in the characteristic solvated form of methyl rehmmanate. In the ultra-violet region lantadene B showed a maximum at 215 $\text{m}\mu$, indicating the presence of an $\alpha\beta$ -unsaturated ester grouping as in rehmmanic acid (λ_{max} 212 $\text{m}\mu$). However lantadene B had a significantly higher absorption (ϵ 18,000) than rehmmanic acid (ϵ 12,400). The infra-red spectra of lantadene B and of rehmmanic acid showed minor, but significant, differences in the finger-print region.

The relation between lantadene B and rehmmanic acid was finally revealed by further degradation. On unimolecular ester pyrolysis, lantadene B methyl ester gave $\beta\beta$ -dimethylacrylic acid and a product which, on catalytic hydrogenation, furnished methyl oleanolate, (III). Vigorous alkaline hydrolysis of lantadene B afforded $\beta\beta$ -dimethylacrylic acid

		Infra-red maxima (cm^{-1})			
		CO_2H	C:O	C:O of C:C-CO-O	C:C of C:C-CO-O
Rehmmanic acid (II; R = angeloyloxy, R' = H)	{ Nujol	1738	1714	1675	1644
	{ CHBr_3	1752	1710	1710	1652
Icterogenin (I; R = angeloyloxy)	Nujol	1740	1700	1678	1643
Lantadene B (II; R = $\beta\beta$ -dimethyl- acryloyloxy, R' = H)	{ Nujol	1736	1715	1678	1653
	{ CHBr_3	1752	1712	1712	1650

identified as the *p*-bromo- and *p*-phenyl-phenacyl esters, and 22 β -hydroxyoleanonic acid (II; R = OH, R' = H) obtained earlier (Barton and de Mayo, *loc. cit.*, p. 900) by similar hydrolysis of rehmmanic acid. The identity of the hydrolysis product was confirmed by its conversion into the methyl ester (II; R = OH, R' = Me) and by oxidation of the latter to methyl 22-oxo-oleanonate (Barton and de Mayo, *loc. cit.*, p. 900). These degradations, coupled with the ultra-violet absorption spectrum (see above), establish the constitution of lantadene B as 22 β -($\beta\beta$ -dimethylacryloyloxy)oleanonic acid (II; R = $\beta\beta$ -dimethylacryloyloxy, R' = H). 22 β -Hydroxyoleanonic acid has been further characterised by conversion into the dibenzoate of methyl 22 β -hydroxyoleanolate.



Louw (*loc. cit.*) has reported that lantadene A affords an acetyl derivative on treatment with acetic anhydride and sodium acetate. Similar reaction of lantadene B has given us the mixed anhydride (II; R = $\beta\beta$ -dimethylacryloyloxy, R' = Ac), characterised by its ready solvolysis with ethanol back to lantadene B. The infra-red spectrum of the mixed anhydride showed bands (in CS_2 solution) at 1822 and 1755 (mixed anhydride), at 1718 and 1222 ($\alpha\beta$ -unsaturated ester), at 1708 (6-ring ketone), and at 1650 cm^{-1} (C:C conjugated with C:O) in agreement with the assigned constitution.

A more thorough investigation of the acidic constituents of *Lantana camara* L. is in hand in an endeavour to determine the nature of lantadene A.

EXPERIMENTAL

For general experimental conditions see Part VII (*J.*, 1952, 2339). Rotations were determined in CHCl_3 solution. Ultra-violet absorption spectra were taken in EtOH solution with the Unicam S.P. 500 Spectrophotometer. Infra-red spectra were kindly determined by Messrs. Glaxo Laboratories. Silica gel for chromatography was obtained from Messrs. Hopkin and Williams Ltd.

Isolation of Crude Lantadene.—The crude lantadene mixture was isolated from a batch of *Lantana camara* leaves exactly as described by Louw (*loc. cit.*). The crude product had m. p. 274—282° (decomp.), $[\alpha]_{\text{D}} +82^\circ$ (*c.*, 2.00), λ_{max} . 211 $\text{m}\mu$ (ϵ 16,900).

Lantadene B.—Chromatography of crude lantadene (4.03 g.) over silica (250 g.) and elution with benzene–ether (19 : 1) gave lantadene B (3.61 g., 90%) in fractions melting between 277° and 290°. Recrystallisation once from benzene–hexane afforded pure *lantadene B*, m. p. 293—294° (decomp.), $[\alpha]_{\text{D}} +85^\circ$ (*c.*, 2.40), λ_{max} . 214 $\text{m}\mu$ (ϵ 18,000) (Found: C, 75.8; H, 9.6. $\text{C}_{35}\text{H}_{52}\text{O}_5$ requires C, 76.05; H, 9.5%), whose physical constants were unchanged on repeated fractional crystallisation and on rechromatography. Authentic lantadene B had m. p. 292—294° (decomp.), undepressed on admixture with the lantadene B described above. *Lantadene B oxime*, prepared in the usual way with pyridine–hydroxylamine hydrochloride, had m. p. (from methanol) 268—269° (decomp.) (Found: C, 74.15; H, 9.45. $\text{C}_{35}\text{H}_{53}\text{O}_5\text{N}$ requires C, 74.05; H, 9.4%). *Lantadene B 2 : 4-dinitrophenylhydrazone* had m. p. (from chloroform–methanol) 264—265° (Found: C, 66.85; H, 7.65. $\text{C}_{41}\text{H}_{56}\text{O}_8\text{N}_4$ requires C, 67.2; H, 7.7%).

Lantadene B Methyl Ester.—Lantadene B (223 mg.) in chloroform (1 ml.) was treated with excess of ethereal diazomethane, and the excess of reagent destroyed with one drop of acetic acid. Chromatography over alumina and elution with benzene gave *lantadene B methyl ester* (174 mg.), m. p. (from hexane) 234—236°, $[\alpha]_{\text{D}} +89^\circ$ (*c.*, 2.00) (Found: C, 76.3; H, 9.8. $\text{C}_{36}\text{H}_{54}\text{O}_6$ requires C, 76.3; H, 9.6%).

Treatment of Lantadene B with Acetic Anhydride.—Lantadene B (133 mg.) with anhydrous sodium acetate (500 mg.) and acetic anhydride (3 ml.) was refluxed for 3 hr., cooled, poured into water, and extracted with chloroform. After being washed with sodium hydrogen carbonate solution, the chloroform solution was evaporated *in vacuo* to give the mixed *anhydride* (142 mg.), m. p. 179—189°. Recrystallised from hexane this had m. p. 190—192°, $[\alpha]_{\text{D}} +81^\circ$ (*c.*, 1.52) (Found: C, 74.4; H, 9.25. $\text{C}_{37}\text{H}_{54}\text{O}_6$ requires C, 74.7; H, 9.15%). The anhydride (50 mg.) in ethanol (5 ml.) was refluxed for 15 min. Concentration of the solution *in vacuo* gave back lantadene B, m. p. and mixed m. p. 293—294° (decomp.).

Pyrolysis of Lantadene B Methyl Ester.—The methyl ester (169 mg.) was pyrolysed at 550/0.5 mm. under the conditions used by Barton and de Mayo (*loc. cit.*, p. 887). The acid evolved in the pyrolysis corresponded to 37% elimination. It had m. p. 64—66° and gave marked depressions in m. p. on admixture with both angelic and tiglic acids, but was undepressed in m. p. on admixture with authentic $\beta\beta$ -dimethylacrylic acid of m. p. 69—70°.

The non-acidic product of the pyrolysis was triturated with hexane and the (unchanged) crystalline lantadene B methyl ester was filtered off. The hexane-soluble material (69 mg.) was filtered through alumina in benzene solution, and the eluted product (43 mg.) was hydrogenated in acetic acid over platinum for 12 hr. The hydrogenation product was refluxed with 5% methanolic potassium hydroxide for 20 min. (to hydrolyse any β -acetate that had been formed) and the product chromatographed over alumina. Elution with benzene–ether (3 : 1) gave methyl oleanolate (m. p. and mixed m. p.).

Hydrolysis of Lantadene B.—Lantadene B (400 mg., 0.725 mmole) in ethanolic potassium hydroxide (20 ml.; 4%) was refluxed for 6 hr. (*cf.* Barton and de Mayo, *loc. cit.*, p. 900). After removal of most of the ethanol *in vacuo*, water (20 ml.) was added and sodium 22 β -hydroxyoleanonate (see below) removed by filtration. Steam-distillation of the acidified (dilute sulphuric acid) filtrate gave 0.675 milliequiv. of steam-volatile acid. Evaporation of the titrated distillate *in vacuo* furnished the sodium salt of the acid. This sodium salt (48 mg.) was converted into the *p*-bromophenacyl ester. Recrystallised from hexane this had m. p. 100.5—101°, undepressed on admixture with an authentic specimen (m. p. 100—100.5°; Smith and Rosenbaum, *J. Amer. Chem. Soc.*, 1951, 73, 3843). The *p*-phenylphenacyl ester, prepared similarly and crystallised from methanol, had m. p. 142—143°, undepressed on admixture with an authentic specimen (Smith and Rosenbaum, *loc. cit.*) of the same m. p.

The sodium salt removed by filtration (see below) was acidified and the product crystallised from aqueous methanol, to give 22 β -hydroxyoleanonic acid, m. p. 233—236° (decomp.), $[\alpha]_{\text{D}} +90^\circ$ (*c.*, 1.55), undepressed in m. p. on admixture with an authentic specimen of m. p. 230—

234° (decomp.), $[\alpha]_D + 92^\circ$ (Barton and de Mayo, *loc. cit.*, p. 900). Methylation with diazomethane furnished methyl 22 β -hydroxyoleanonate, m. p. (from methanol after drying *in vacuo* at 95°) 188—189°, $[\alpha]_D + 86^\circ$ (*c.*, 2.06), undepressed in m. p. on admixture with an authentic specimen (Barton and de Mayo, *loc. cit.*, p. 900). Oxidation of this ester (79 mg.) in "AnalaR" acetic acid (3 ml.) with chromium trioxide (18 mg.) in 2 drops of water overnight at room temperature gave methyl 22-oxo-oleanonate, m. p. (from methanol) 204—206°, $[\alpha]_D + 40^\circ$ (*c.*, 2.38), undepressed in m. p. on admixture with an authentic specimen (Barton and de Mayo, *loc. cit.*, p. 900).

Methyl 22 β -Benzoyloxyoleanolate Benzoate.—A solution of 22 β -hydroxyoleanonic acid (52 mg.) in "AnalaR" acetic acid (6 ml.) was hydrogenated overnight with a platinum catalyst. The product was methylated with diazomethane and then refluxed with methanolic potassium hydroxide (5%; 5 ml.) for 15 min. The resulting methyl 22 β -hydroxyoleanolate in dry pyridine (1 ml.) was treated with redistilled benzoyl chloride (62 mg.) and left at room temperature for 4 days. Chromatography over alumina and elution with benzene gave *methyl 22 β -benzoyloxyoleanolate benzoate* (37 mg.), m. p. (from chloroform-methanol) 210.5—212°, $[\alpha]_D + 60^\circ$ (*c.*, 1.87), λ_{\max} . 229 m μ (ϵ 29,700) (Found: C, 78.0; H, 8.35. C₄₆H₅₈O₆ requires C, 77.75; H, 8.4%).

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