SPECIALIA

Triterpenes of Toxic and Non-Toxic Taxa of *Lantana camara*

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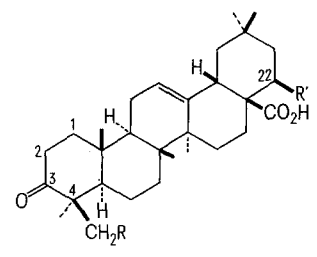
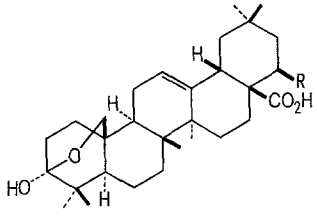
Summary. The taxa of *Lantana camara* toxic to animals contain lantadene A and lantadene B, whereas in two non-toxic taxa other triterpenes predominate. Several new triterpenes have been characterized. Contrary to earlier claims, lantadene A and to a lesser extent lantadene B are toxic when administered intraruminally to sheep.

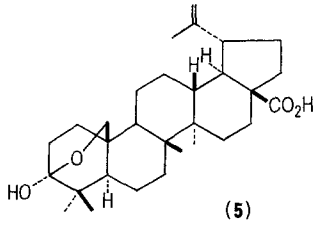
Of some 29 taxa of *Lantana camara* L. (Verbenaceae) found in Australia[1], 9 have assumed pest proportions and 8 of these, with the exception of the widespread 'Common Pink', are toxic to livestock. Lantadene A **1** and lantadene B **2** have been isolated from *L. camara*[2,3], and from an Indian *L. camara*[4,5] lantanolic acid **3** and lantic acid which are oleanane and ursane derivatives respectively and have an oxide bridge from C(25) to C(3) and a hemiketal system at C(3). There has, however, been no previous comparison of the chemical differences between taxa, and contradictory claims have been made earlier about the animal toxicity of lantadene A.



(1) R = H; R' = O·CO·C(Me)=CH(Me)
(2) R = H; R' = O·CO·CH=C(Me)(Me)
(4) R = H; R' = OH
(9) R = OH; R' = O·CO·C(Me)=CH(Me)
(10) R = OH; R' = H



(3) R = H
(6) R = O·CO·C(Me)=CH(Me)
(7) R = O·CO·CH=C(Me)(Me)
(8) R = OH



(5)

Material and methods. Collections of leaf and stem samples were made from a number of taxa known to be toxic from earlier feeding trials, and from the non-toxic taxa 'Common Pink' and 'Townsville Prickly Orange'. Separation of the triterpenoid constituents by high pressure liquid chromatography on Kieselgel 60 revealed the presence of several new triterpenes. The toxicity of various triterpenes in sheep was determined by intraruminal administration in DMSO solution.

Results and discussion. All the toxic taxa now examined contained lantadene A and lantadene B. In 'Pink-edged Red' lantadene A and lantadene B in the approximate ratio 2:1 make up approximately 2.2% of the dry weight of the plant. Also present are relatively small amounts of the corresponding 3β-alcohols, identical with the products obtained by reducing lantadene A and lantadene B with sodium borohydride, and 22β-hydroxy-3-oxoolean-12-en-28-oic acid (**4**), none of which were previously known to occur in *L. camara*. Oleanolic acid and oleanonic acid are also present. 'Townsville Red-centred Pink' also has a high content of lantadene A and lantadene B, but differs in having some betulonic and betulic acid, whereas 'Helidon White' has a relatively low content (0.5%) of lantadene A and lantadene B and has betulonic acid (0.7%) as the major constituent.

'Common Pink' contains no lantadene A or lantadene B, and apart from small amounts of betulic acid, betulonic acid, and oleanolic acid, the triterpenes of this taxon differ markedly from those of all other taxa examined in having an oxide bridge from C(25) to C(3) and a hemiketal system at C(3). The two previously known triterpene acids of this type, lantanolic acid (**3**) and lantic acid[4,5], both occur in 'Common Pink'. A new constituent is the analogue of lantanolic acid and lantic acid in the lupane series, lantabetulic acid (**5**), $C_{30}H_{46}O_4$, and its structure has been established by conversion into methyl lup-20(29)-en-28-oate by a reaction sequence similar to that used in establishing the structure of lantanolic acid[4]. Two other new acids [both $C_{35}H_{52}O_6$],**6** and **7**, isolated as a mixture, have been characterized spectroscopically as the corresponding C(25), C(3)-oxide bridged analogues of lantadene A and lantadene B, and are converted on hydrolysis into 22β-hydroxylantanolic acid (**8**) (methyl ester, $C_{31}H_{48}O_5$, m.p. 232–234°, $[\alpha]_D$ + 100° in $CHCl_3$), the structure of which has been determined by a detailed spectroscopic examination (MS, NMR).

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[3] D. H. R. BARTON, P. DE MAYO, F. W. WARNHOFF, O. JEGER and G. W. PEROLD, J. chem. Soc. *1954*, 3689.
[4] A. K. BARUA, P. CHAKRABARTI, S. P. DUTTA, D. K. MUKHERJEE and B. C. DAS, Tetrahedron *27*, 1141 (1971).
[5] A. K. BARUA, P. CHAKRABARTI, P. K. SARIYAL and B. C. DAS, J. Indian chem. Soc. *46*, 100 (1969).

'Townsville Prickly Orange' is not a pest taxon and has been found to be non-toxic in feeding trials. It is the only taxon in which C(24)-hydroxylated triterpenes have been found. Although lantadene A and lantadene B are present they are minor constituents, while oleanonic acid and 3-oxours-12-en-28-oic acid predominate. This taxon also contains icterogenin (**9**), previously obtained from *Lippia rehmanni*[6], and a new triterpene acid (methyl ester, $C_{31}H_{48}O_4$, m.p. 212–214°, $[\alpha]_D$ + 108° in $CHCl_3$) shown to be 24-hydroxy-3-oxoolean-12-en-28-oic acid (**10**). The methyl ester of this acid undergoes elimination of formaldehyde in a retroaldol reaction with dilute methanolic sodium hydroxide solution to give the known methyl hedragonate, and it is thereby shown to be the C(4) epimer of methyl 23-hydroxy-3-oxoolean-12-en-28-oate which has also been converted into methyl hedragonate[6].

Although it is not known to what extent the differences in toxicity between the taxa depend on the nature of the triterpenes and on their overall yields, these observations provide a basis for explaining the results of feeding trials. Lantadene A administered intraruminally as a single dose of 80 mg/kg body weight to sheep produces the characteristic toxicity previously shown to occur after feeding the whole *Lantana* plant[7,8]. From a study[9] of biliary secretion in the rabbit it had been concluded that when icterogenin, lantadene A and lantadene B were administered as fine aqueous suspensions into the peritoneal cavity, only icterogenin was active, and that any activity shown by samples of lantadene A was due to trace quantities of the toxic 3β-alcohol that would also be present with it. It was further suggested[10] that since lantadene A was non-toxic to the rabbit in these studies, the toxicity previously attributed by LOUW[11] and SEAWRIGHT[7] to lantadene A in oral dosing experiments in sheep was due to the unsuspected presence of small amounts of the 3β-alcohol. When the latter is dosed intraruminally to sheep at 3 mg/kg body weight, the amount estimated to be present in an effective dose of toxic *Lantana* leaf, however, no poisoning results. The present studies thus support the original observation by LOUW[11] that the toxicity to sheep of the crystalline isolate from *Lantana* leaves was due to the presence of the lantadene A itself, and further that the 3β-alcohol, in the amount likely to be present, is not sufficiently toxic when taken by this route for it to contribute significantly to the toxicity of the plant in the field.

Lantadene B in intraruminal doses of 200 to 300 mg/kg body weight was found also to be icterogenic for sheep and caused toxicity equivalent in severity to that produced by 80 mg/kg of lantadene A and 40 mg/kg of the 3β-alcohol from lantadene A. LOUW[11] found that when 2 g doses of lantadene A and lantadene B respectively were administered orally to adult sheep, lantadene A was toxic while lantadene B was not. As lantadene B differs from lantadene A only in the esterifying acid at C(22) it was accordingly concluded[9] that the angeloyloxy group at C(22) was a necessary structural requirement for icterogenicity. The present studies suggest that the dose rates of lantadene B used formerly[10,11] were too low to produce a toxic effect in those animal experiments. Lantadene B is however often a major constituent of *Lantana* leaves, and could thus contribute significantly to the overall toxicity of the plant.

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[7] A. A. SEAWRIGHT, Aust. vet. J. *39*, 340 (1963).
[8] A. A. SEAWRIGHT, Pathology Vet. *1*, 504 (1964).
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Synthesis of Ovulation-Inhibiting Compounds; Structure-Activity Relationship

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Summary. We describe the synthesis of some new derivatives of benzo(4,5)cyclohepta(1,2-b)thiophene which inhibit ovulation and the secretion of luteinizing hormone (LH) in the rat. We also describe the relationship between the structure and activity of these compounds.

In connection with a study on the ovulation and LH-inhibiting effects in rats of a particular benzocycloheptathiophene derivative: compound 26–921[1], we wish to report the preparation of 10-substituted benzo(4,5)cyclohepta(1,2-b)thiophenes from type **3**.

In another report[2], we describe the synthesis of 10-keto derivatives from type **1**. Reaction of **1** with phenyl magnesium bromide in anhydrous tetrahydrofuran or better phenyl lithium in anhydrous ether at room temperature for 1 h and at reflux for 1 additional h, followed by dehydration of the obtained hydroxycompounds **2** in a mixture of hydrochloric acid and isopropanol gave **3** (9,10: double bond; R′ = phenyl, R = alkyl, e.g. methyl).

On the other hand, **1** failed to react with alkyl magnesium halogenides, and when the reaction with methyl lithium was carried out at −20°, a very small yield of the desired alcohol **2** was obtained.

The second approach involved the condensation of the alkyl 2-thienyl ketones **5** with the diethyl *o*-cyanobenzylphosphonate in N,N-dimethylformamide at 20–100° for 2–5 h, to produce the compounds **6**, which were hydrogenated in ethanol with palladium on charcoal at 100° and 20 at. and hydrolyzed with potassium hydroxide in methyl diglykol at 150–180°. The obtained benzoic acid derivatives **7** were cyclized with polyphosphoric acid at 80–100° for 10–30 min to the ketones **8** (9,10: single bond; R′ = alkyl).

In the preparation of the final compounds listed partially in the Table, the attachment of the side chains in 4 position of the tricyclic intermediates and the following

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[2] E. WALDVOGEL, G. SCHWARB, J. M. BASTIAN and J. P. BOURQUIN, Helv. chim. Acta, in press (1976).