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OPTIMIZATION OF LANTADENES ISOLATION AND PREPARATION OF 22β-HYDROXYOLEANONIC ACID

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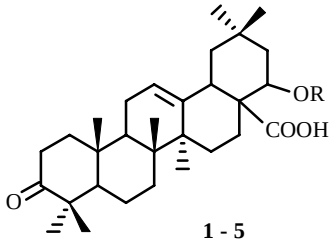
The lantadenes fraction from Lantana camara *was isolated and transformed to the medicinally important intermediate 22*β*-hydroxyoleanonic acid. The compound was studied for hepatotoxicity using lantadene A as standard and found to be nontoxic.*

Key words: lantadenes, 22β-hydroxyoleanonic acid, hepatotoxicity.

Lantana (*Lantana camara* L.), commonly known as red or wild sage, is one of the ten most noxious weeds in the world [1]. It has encroached upon vast expanses of land area including pastures, orchards, tea gardens forests, and agricultural lands in tropical and subtropical parts of the world and poses a great threat to grazing livestock and overall ecological balance [2, 3]. Attempts to control this weed using mechanical, chemical, and biological methods have met with limited success and, therefore, there is a need to find some novel approaches for the utilization of this plant as a resource.



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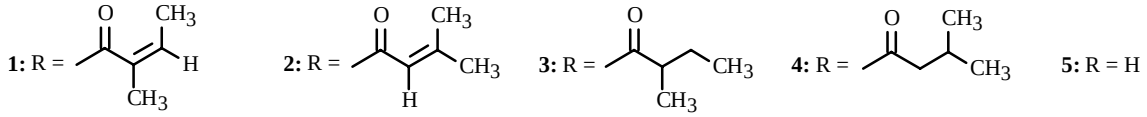

A number of medicinal properties have also been reported of different parts of lantana, and during the past few years various research groups reported a number of chemical compounds from this plant, the majority of which are triterpenoids, including lantadenes (**1**–**4**) [2, 4]. Recently, lantadenes and other triterpenoids from this plant have been found to exhibit a wide spectrum of pharmacological activities, including antitumor effects. These observations indicate that lantadenes have the potential to be developed as an antitumor therapeutic agent [5, 6]. These compounds differ in the structure of the side chain attached at the C-22 position through an ester linkage, and there are indications that these structural variations may play an important role in their pharmacological activity. However, many of theses compounds are present in very small amounts and due to very little difference in their physicochemical properties, their isolation in sufficient amounts is difficult. One promising approach to obtaining these compounds is through the semisynthetic procedure using 22β-hydroxyoleanonic acid (**5**) as the starting material, which can be obtained by the hydrolysis of semipurified lantadenes. In this paper we report on the optimization of the isolation procedure for the partially purified lantadenes fraction, as well as lantadene A (LA) and their transformation to 22β-hydroxyoleanonic acid by hydrolysis.

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TABLE 1. Toxicity Sudies of 22β-Hydroxyoleanonic Acid Using LA as a Standard

Bilirubin (mg/100 ml)	SGOT (U/L)	SGPT (U/L)
	Group treated with LA	
7.02	664.1	305.4
10.04	698.4	302.2
9.84	696.2	304.1
8.06	696.6	309.2
8.74±2.5[a]	696.3±3.1[a]	305.2±3.9[a]
	Group treated with 22β-hydroxyoleanonic acid	
0.71	46.2	38.6
0.69	46.7	39.7
0.70	45.2	39.4
0.60	46.6	38.6
0.67±0.001[b]	46.1±0.4[a]	39.0±0.3[a]
	Group treated as control	
0.76	46.2	34.2
0.68	41.4	33.8
0.72	43.7	35.7
0.62	40.7	35.4
0.69±0.003[b]	42.8±5.8[a]	34.7±0.8[a]

Values are mean + SD.
[a]$p \leq 0.01$; [b]$p \leq 0.001$.
There are four animals in each group. The animals were killed after 48 hours of dosing.

The lantadenes fraction was isolated by suspending the lantana leaf extract in a methanol-water mixture and extraction with chloroform. The yield obtained by this method was 1.06%, which is higher compared to earlier reports [7]. For the isolation of LA, the lantadenes fraction was chromatographed on silica gel column and the yield obtained was 45%. The partially purified lantadenes fraction was directly hydrolyzed by refluxing with alcoholic potassium hydroxide to obtain **5**, and structure of this compound was confirmed by IR, PMR, and ^{13}C NMR spectra. ^{13}C NMR spectrum of the compound supports a triterpene structure with seven methyl groups, one trisubstituted double bond, and two carbonyl groups. Absorption at δ 180.5 and 76.6 were due to the C-28 carbonyl carbon and of C-22 attached to the O-H group respectively. The spectrum showed a downfield shift of the C-3 keto group which was resonated at δ 217.6. The number of primary, secondary and tertiary carbons was confirmed by the DEPT technique. The ^{1}H NMR spectrum of this compound beside other peaks exhibited signals for seven tertiary methyl groups at δ 0.85 (3H), 0.89 (3H), 1.04 (6H), 1.09 (3H), 1.11 (3H), and 1.15 (3H) as singlets. A signal for the trisubstituted double bond at C-12 appeared at δ 5.45 (1H, broad). A signal of the OH group at the 22 position appeared at δ 4.8 (broad), which disappeared on D_2O exchange. Various lantadenes including LA have been found to be hepatotoxic in various animals and it has been speculated that the side chain attached at the C-22 position may be responsible for this hepatotoxicity. To test this, hepatotoxic studies of **5** were carried out on guinea pigs using LA as a standard. In these studies, compound **5** has been found to be nontoxic. This compound is an important intermediate that can be used for the semisynthesis of a series of lantadenes with potential antitumor activities.

EXPERIMENTAL

Leaves of *Lantana camara* var. *aculeate* (red flower variety) were collected from Palampur (HP, India), and a fine powder was prepared. ^{13}C NMR and PMR spectra were recorded on a Bruker AM-300 spectrometer (75.5 and 300 MHz, respectively) in $CDCl_3$ using $SiMe_4$ as internal standard. IR spectra were recorded on a Perkin Elmer RX-1 spectrometer. Melting points were determined on Boetius stage apparatus and were uncorrected.

Extraction and Isolation of Lantadenes. To 100 g of lantana leaf powder, 500 mL methanol was added and the mixture kept for 24 h with intermittent shaking. The extract was separated by filtration through muslin cloth and decolorized with 20 g of activated charcoal which gave a golden yellow color to the extract [8]. The solvent was removed under reduced pressure and the residue was suspended in a methanol–water (1:7) mixture and extracted with chloroform (2×15 mL). The organic layer was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. The solid residue obtained was recrystallized from methanol to obtain partially purified lantadenes (1.06 g, 1.06%) as white crystalline product. For isolation of LA, partially purified lantadene fraction (1.0 g) was chromatographed over silica gel column (30 g, 60–120 mesh) using chloroform and chloroform–methanol (99.5:0.5) as eluting solvent. The LA enriched fractions were pooled, the solvent was removed *in vacuo*, and the solid residue was recrystallized twice from methanol to obtain pure LA (0.45 g) as white crystals, R_f 0.7 ($CHCl_3$ –CH_3OH; 9.8:0.2), mp 283°C (285–288°C) [9].

Preparation of 22β-Hydroxyoleanonic Acid. To 1g of partially purified lantadenes fraction, 170 mL of ethanolic potassium hydroxide solution (10% w/v) was added and the reaction mixture was refluxed for 6 h. The solvent was removed *in vacuo* and the residue was diluted with water (15 mL). The mixture was acidified with conc. HCl to pH 1-2 and extracted with ether (3×15 mL). The combined ethereal layer was washed with sodium carbonate (4% w/v, 3×15 mL) and water (15 mL) and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue was recrystallized from a methanol:water mixture to obtain 22β- hydroxyoleanonic acid as white crystals (0.45 g, 54.4 %), mp 234°C (234–236°C) [8].

IR (ν_{max}, KBr, cm^{-1}): 3590 (OH stretch), 3480 (OH stretch of –COOH), 1720 (C=O, 3-keto).

PMR ($CDCl_3$, δ, J/Hz): 0.85 (3H, s, CH_3), 0.89 (3H, s, CH_3), 1.04 (6H, s, $2CH_3$), 1.09 (3H, s, CH_3), 1.11 (3H, s, CH_3), 1.15(3H, s, CH_3), 3.05 (1H, dd, J = 3.7, C-18-H), 3.5 (1H, s, OH), 3.93 (1H, t, J = 3, C-22- H), 4.8 (1H, broad, C-22-OH), 5.45 (1H, s, C-12-H).

^{13}C NMR ($CDCl_3$, δ): 39.3 (C-1), 34.2 (C-2), 217.6 (C-3), 47.5 (C-4), 55.4 (C-5), 19.5 (C-6), 32.3 (C-7), 39.2 (C-8), 46.9 (C-9), 36.8 (C-10), 24.4 (C-11), 121.2 (C-12), 143.3 (C-13), 42.0 (C-14), 27.7 (C-15), 23.6 (C-16), 52.2 (C-17), 39.1 (C-18), 45.8 (C-19), 30.1 (C-20), 38.1 (C-21), 76.6 (C-22), 26.5 (C-23), 21.5 (C-24), 15.1 (C-25), 16.7 (C-26), 25.8 (C-27), 180.5 (C-28), 33.8 (C-29), 26.8 (C-30).

Hepatotoxicity Studies of 22β-Hydroxyoleanonic Acid. The hepatotoxicity studies of 22β-hydroxyoleanonic acid were carried out using LA as standard. Adult female guinea pigs were starved overnight and divided at random into three groups A, B, and C, consisting of four animals each. Animals in group A were administered LA orally as a single dose filled in a gelatin capsule at a level of 125 mg / kg body weight [10]. Animals in group B were similarly administered 22β-hydroxyoleanonic acid orally at a level of 125 mg/kg of body weight. Animals in group C were used as control and administered empty gelatin capsule shells. The effect of LA and 22β-hydroxyoleanonic acid on the guinea pigs is shown in Table 1.Total bilirubin, SGOT, and SGPT were determined in blood plasma [11]. The animals were sacrificed after 48 hours of dosing.

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