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A BIOACTIVE TRITERPENE FROM *LANTANA CAMARA*

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Key Word Index—*Lantana camara*; Verbenaceae; 22β-acetoxylantic acid; 22β-acetyl lantoic acid; antimicrobial; antimutagen.

Abstract—*Lantana camara* afforded a novel triterpene 22β-acetoxylantic acid and the known triterpenes, lantic acid, 22β-dimethylacryloyloxylantanolic acid, a mixture of 22β-dimethylacryloyloxy lantanolic acid and 22β-angeloyloxylantanolic acid and lantanolic acid. 22β-Acetoxylantic acid showed antimicrobial activity against *Staphylococcus aureus* and *Salmonella typhi*. This compound and 22β-dimethylacryloyloxy lantanolic acid also showed antimutagenic activity.

INTRODUCTION

Lantana camara is a native of tropical America, but now occurs throughout the Philippines in thickets and waste lands at low and medium altitude [1]. Crushed leaves of *L. camara* are applied as a poultice to wounds and injuries, while the decoction of boiled leaves are used for washing affected areas in cases of dermatitis, eczema, tinea and furuncles. The antipyretic properties of the leaves are mainly attributed to lantadene A [2]. Previous investigations of *L. camara* afforded triterpenes of the lantadene type [3–6], lantanolic acid and lantic acid [5], lantoic acid [7], icterogenin and hederagonic acid [8], camaroside and phenolic compounds [9]. We now report the isolation of a new triterpene and describe its antimicrobial and antimutagenic properties.

RESULTS AND DISCUSSION

The chloroform extract of the air dried leaves of *L. camara* afforded a novel triterpene (**1**), and the known lantic acid (**2**), 22β-dimethylacryloyloxylantanolic acid (**3**), a mixture of **3** and 22β-angeloyloxylantanolic acid (**4**) and lantanolic acid (**5**). The structure of **1** was elucidated by NMR (^{1}H, ^{13}C, DEPT, COSY) and FT-IR spectroscopy and mass spectrometry.

The mass spectrum showed a molecular ion peak at *m/z* 528, which corresponds to a molecular formula of $C_{32}H_{48}O_6$. The peak at *m/z* 468 resulted from the loss of acetic acid, while that at *m/z* 450 resulted from the loss of acetic acid and water. This suggested that **1** was a triterpene with acetate and alcohol functionalities. The ^{1}H NMR spectrum of **1** indicated an olefinic hydrogen at δ 5.39 (1H, *s*, *br*), a proton on an ester bearing carbon at δ 5.00 (1H, *s*, *br*) and two hydrogens on a carbon singly bonded to an oxygen at δ 4.21 and 3.90 (each 1H, *d*, $J = 9$ Hz). The ester functionality of **1** was confirmed by the FT-IR absorption band at 1250 and 1720 cm^{-1}. The spectrum also indicated seven methyl groups, one of which belonged to an acetate (Table 1). In view of the fact that all known triterpenes from *Lantana camara* belong to either the ursolic or oleanolic acid families, such a structure was considered likely for compound **1**.

The COSY correlations were consistent with an ursolic acid skeleton with oxygenated substituents at C-3, C-25 and C-22. The COSY spectrum indicated the following isolated spin systems: the hydrogen at δ 5.00 (H-22) was coupled to the methylene protons at δ 1.47 and 1.79. The latter signal coupled to δ 1.35, which was in turn coupled to the methyl protons at δ 0.90 (*d*, $J = 7$ Hz, 29-CH_3). The signal at δ 1.35 was also coupled to δ 1.52, which was in turn coupled to another methyl doublet at δ 0.92 (*d*, $J = 7$ Hz, 30-CH_3). An olefinic hydrogen at δ 5.35 (H-12) was coupled to the allylic methylene protons at δ 1.79 and 2.10, with the latter coupled to δ 1.23. Two isolated sets of coupled methylene protons were observed: one at δ 2.40 was coupled to δ 1.78 and to the methylene protons at δ 1.35 and δ 0.84; while another at δ 2.14 and δ 1.70 was coupled to δ 1.18 and δ 1.52. The hydrogen at δ 1.50 (H-5) was coupled to the methylene

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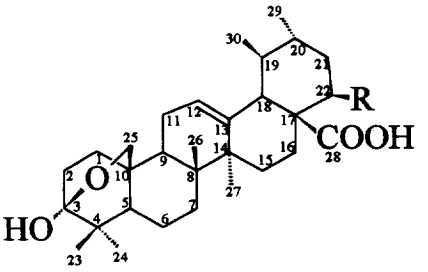

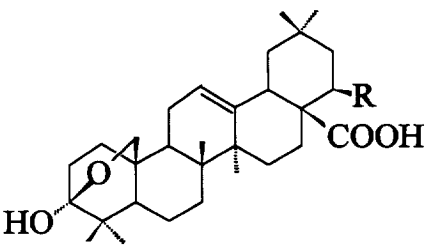

1 R = $OCOCH_3$
2 R = H

3 R = $OCOCH{=}C(CH_3)_2$
4 R = OAng
5 R = H

protons at δ 1.34 and 1.20, which were in turn coupled to δ 1.63. The methylene protons (25-H_2) at δ 4.21 and δ 3.45 correlated only with each other.

Further information was given by the ^{13}C and DEPT NMR spectra of **1** which indicated the following functionalities: a carbonyl of an ester at δ 170.2, a carbonyl of a carboxylic acid at δ 178.5 (IR 3400 cm^{-1}), an acetal at δ 98.9 (IR 1020 cm^{-1}), an oxygenated methylene at δ 67.9, an oxygenated methine at δ 75.7, a fully substituted olefinic carbon at δ 137.2, a methine olefinic carbon at δ 126.1, eight methylenes and seven methyls (Table 2). Thus, compound **1** contained 32 carbons, 46 hydrogens attached to carbons, a hydrogen of a carboxylic acid, a hydrogen of a hydroxyl and six oxygens, in agreement with the mass spectral data. From the molecular formula, the index of hydrogen deficiency is nine. With three double bond equivalents (2 C = O and 1 C = C) deduced from the ^{13}C and DEPT NMR spectra of **1**, the remaining hydrogen deficiency confirmed the presence of six carbocyclic rings.

Correlation of the partial structures deduced from the COSY spectrum and the data from the ^1H, DEPT, ^{13}C NMR, FT-IR and mass spectra resulted in structure **1**. This was confirmed by the isolation of known triterpenes (**2**–**5**) of similar structures. The proposed name of **1** is 22β-acetoxylantic acid. This may also be named 22β-acetyl lantoic acid as it is the 22-acetate derivative of lantoic acid [7].

The structures of **3**–**5** were determined by comparing their ^1H NMR spectral data with those of **1** (Table 1). Compounds **3**, **4** and **5** have been reported as constituents of *L. camara* (common pink), but this is the first time that **3** has been obtained pure [5].

Lantana camara is known to have antimicrobial properties. Thus, compound **1** was tested for its antimicrobial potential, and the results of the study are presented in Table 3. Among the six microorganisms tested, compound **1** was found to be active against *S. aureus* and *S. typhi* with an average antimicrobial index of 0.95 and 0.55 respectively, at a concentration of 30 μg. For the antibiotics chloramphenicol for *S. aureus*, and tetracycline for *S. typhi*, the average antimicrobial index are 1.6 and 0.8, respectively, at a concentration of 30 μg.

The antimutagenicity potential of compounds **1** and **3** were also tested by the use of the Micronucleus test. Results of the study shown in Table 4 indicated that at a dosage of 6.75 mg kg $mouse^{-1}$, compound **1** reduced the number of micronucleated polychromatic erythrocytes (MPCE) induced by Mitomycin C by 76.7%, while compound **3** reduced the MPCE by 60.0%. Statistical analysis using the *t*-test showed that there is a significant decrease in MPCE at $\alpha = 0.01$.

Table 1. ^1H NMR spectral data of compounds **1**–**4** (300 MHz) in $CDCl_3$

Chemical shifts, δ					
1	**2**	**3**	**4**	**5**	Functionalities
5.39 (*s*, *br*)	5.29 (*s*, *br*)	5.39 (*s*, *br*)	5.39 (*s*, *br*)	5.29 (*s*, *br*)	C=CH
4.95 (*s*, *br*)		5.00 (*s*, *br*)	5.00 (*s*, *br*)		CH—O (ester)
4.21 (*d*, *J* = 9 Hz),	4.25 (*d*, *J* = 9 Hz),	4.21 (*d*, *J* = 9 Hz),	4.21 (*d*, *J* = 9 Hz),	4.21 (*d*, *J* = 9 Hz),	CH_2O
3.90 (*d*, *J* = 9 Hz)	3.90 (*d*, *J* = 9 Hz)	3.90 (*d*, *J* = 9 Hz)	3.90 (*d*, *J* = 9 Hz)	3.90 (*d*, *J* = 9 Hz)	
1.14 (*s*), 1.08 (*s*),	1.14 (*s*), 1.08 (*s*)	1.18 (*s*), 1.08 (*s*),	1.25 (*s*), 1.16 (*s*)	1.22 (*s*), 1.15 (*s*),	CH_3
1.01 (*s*), 0.92	1.01 (*s*), 0.95	1.04 (*s*), 1.00 (*s*),	1.04 (*s*), 1.00 (*s*),	1.10 (*s*), 1.05 (*s*),	
(*d*, *J* = 7 Hz), 0.90	(*d*, *J* = 7 Hz), 0.85	0.92 (*s*), 0.79 (*s*)	0.90 (*s*), 0.80 (*s*)	0.96 (*s*), 0.78 (*s*)	
(*d*, *J* = 7 Hz), 0.73 (*s*)	(*d*, *J* = 7 Hz), 0.75 (*s*)				
2.00 (*s*)					$CH_3C{=}O$
		2.15 (*s*), 1.88 (*s*),			$(CH_3)_2C{=}$
		5.60 (*s*, *br*)			
			6.00 (*m*),		CH=
			2.15 (*s*, *br*),		$=CCH_3$
			1.90 (*s*, *br*)		

Table 2. Comparison of the ^{13}C NMR spectra of compounds **1**–**3** in $CDCl_3$

1 (ppm)	**3*** (ppm)	Functionalities
178.5	178.0	COOH (acid)
170.2	163.4	C=O (ester)
137.2	143.0, 116.0	C=
126.1	122.5, 157.0	CH=
98.9	98.9	O—C—O
67.9	67.7	CH_2—O
75.7	75.3	CH—O
49.3, 42.4, 41.9, 40.2, 39.2		C
50.2, 49.3, 41.9, 39.2, 32.2		CH
35.1, 34.9, 31.2, 29.8, 27.9, 24.8, 23.8, 19.6		CH_2
16.9, 17.7, 18.4, 20.7, 21.2, 23.1, 27.1		CH_3

*The shielded resonances are not indicated because a DEPT spectrum was not obtained for **3**.

Table 3. Antimicrobial index

Bacterium	**1**	Chloramphenicol	Tetracycline	Acetone
E. coli	0.2, 0		1.5, 1.5	
S. aureus	0.7, 1.2	1.5, 1.7		
P. aeruginosa	0.1, 0.2		0.6, 0.5	0.1, 0.1
S. typhi	0.4, 1.0		0.8, 0.8	0.1, 0.2
Fungus		Clotrimazole		
C. albicans	0.2, 0.2	4.0, 3.0		0.2, 0.2
T. mentagrophytes	2.3, 2.3	5.0, 4.0		2.0, 2.1

Table 4. Effects of compounds **1** and **3** on the formation of micronucleated polychromatic erythrocytes (MPCE) induced by Mytomycin C

Compound tested	Dosage (mg kg^{-1})	Ave. no. MPCE/1000 PCE $\pm$ σ*	% reduction in MPCE
1	6.75	1.42 $\pm$ 0.49	76.7%
3	6.75	2.40 $\pm$ 1.25	60.0%
Mitomycin C + DMSO	2.0 mg kg^{-1} 7.5 ml kg^{-1}	6.0 $\pm$ 1.7	

*Average of 15 slides.

Therefore, compounds **1** and **2** possess high antimutagenic activity.

EXPERIMENTAL

Silica gel type 60 (Merck) was used for CC and plastic-backed plates coated with silica gel F254 for TLC. Plates were visualized by spraying with vanillin–H_2SO_4 and warming.

Extraction and isolation. Lantana camara was collected in flower and at fruiting from the University of the Philippines at Los Banos in March. It was identified by Noe Gapas of the Philippine National Museum and deposited at the Philippine National Herbarium (PNH 161774).

The air-dried leaves (600 g) were soaked in $CHCl_3$ (3.6 l) for 3 days to afford a crude extract (23 g). The extract (4 g) was chromatographed on a gravity column packed with dry silica gel (70–230 mesh) using increasing proportions of Me_2CO in $CHCl_3$ (5% increment) and MeOH in Me_2CO (10% increment) as eluents. The 10–20% MeOH in Me_2CO fractions were rechromatographed in 7 : 1.5 : 1.5 (dichloromethane–Et_2O–acetonitrile) as eluent to afford **1** [colorless crystals, mp 270–273°, $\alpha_D = +87.72°$ ($CHCl_3$, *c* 0.057), 30 mg (0.005%)]. The 10–20% Me_2CO in $CHCl_3$ fractions were rechromatographed in 10% Me_2CO^- in $CHCl_3$ to afford **3** [crystals, mp 285–287°, $\alpha_D = +115.38°$ ($CHCl_3$, *c* 0.065), 33 mg (0.0055%)]. The 30–40% Me_2CO in $CHCl_3$ fractions were rechromatographed in 15% Me_2CO in $CHCl_3$ to afford a mixture of **3** and **4**. The 30–50% MeOH in Me_2CO fractions were rechromatographed in 30% Me_2CO in $CHCl_3$ to afford **5** [crystals, mp 307–309°, 21.7 mg (0.0036%)] and **2** [crystals, mp 256–258°, 18.5 mg, (0.0031%)]).

Antimicrobial test. A microbial suspension containing approximately 10^7 cells ml^{-1} was prepared for each test organism for 24-hr agar culture using 0.1% peptone in water. One-tenth (0.1 ml) of the bacterial suspension was transferred into pre-poured 30 ml deep nutrient agar plate, the yeast suspension into glucose yeast peptone agar plate and the fungal suspension on potato dextrose agar plate. About 5 ml of the corresponding melted agar cooled to about 45° was immediately poured into the plate. The plate was swirled to distribute the microbial cells evenly on the plate. After the overlay agar had solidified, three 1-cm dia. holes were cut from equidistant points using a sterile cork borer.

One-tenth (0.1 ml) portions of the extract were placed in duplicate holes per organism. A similar volume of Me_2CO and of the corresponding antibiotic for each test organism was placed in the remaining two wells on the plate. Plates were incubated at room temp. To prevent evaporation of liquid on the petri lid that may cause interference in distribution of organisms on the surface. Bacterial and yeast plates were read after 24 h, while the mold plate was read after 3 days. Clearing zones were measured in millimeters (mm), the average for each supernatant was taken and the antimicrobial activity index (AI) was computed as the mm clearing zone minus mm hole divided by mm hole.

Micronucleus test. Mitomycin C (2.75 mg kg $mouse^{-1}$) and the test compounds (6.75 mg kg $mouse^{-1}$) dissolved in dimethylsulfoxide (DMSO) (7.5 ml kg $mouse^{-1}$) were administered orally to mice of the Swiss strain (7–12 weeks from DOST). For the positive control, Mitomycin C (2.75 mg kg $mouse^{-1}$) and DMSO (7.5 ml kg $mouse^{-1}$) were administered orally to mice of the Swiss strain. For each isolate and control, five mice were tested. The second administration was carried out after 24 hr. Six hr after the second administration, the mice were sacrificed and blood from the bone marrow was flushed with fetal calf serum. Smears on the slide, three per mouse, were prepared. The slides were stained with undiluted May–Gruenwald solution, followed by 50% May–Gruenwald solution, then 15% Giemsa stain [10]. The number of micronucleated polychromatic erythrocytes (MPCE) per 1000 polychromatic erythrocytes (PCE) were then counted with the use of a high power microscope, and results are given as % reduction in MPCE.

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REFERENCES

1. Bonner, W. D., *Plant Biochemistry*. Academic Press, NY, 1950, p. 384.
2. Co, L., *Common Medicinal Plants of the Cordillera Region*. Chestcore, Quezou City, 1989, p. 63.
3. Louw, P. and Onderstepoort, G., *Journal of Veterinary Science and Animal Industry*, 1943, **18**(1–2), 197.
4. Barton, D., de Mayo, R. and Orr, J., *Journal of the Chemistry Society*, 1956, 4160.
5. Barua, A., Sanyal, P., Das, B. and Charbabarti, K., *Journal of the Indian Chemistry Society*, 1969, **46**, 100.
6. Barua, A. K., Chakrabarti, P., Sanyal, P. K., Basu, K. and Nag, K., *Journal of the Indian Chemistry Society*, 1972, **49**(10), 1063.
7. Sarmila, R. and Barua, A. K., *Phytochemistry*, 1985, **24**(7), 1607.
8. Sharma, O., *Journal of Scientific and Industrial Research*, 1989, **48**, 471.
9. Hart, N. and Suares, A., *Australian Journal of Chemistry*, 1976, **29**, 655.
10. Schmid, W., *Chemical Mutagens*, 1976, **4**, 32.