Phytochemistry, 1977, Vol. 16, pp. 1845–1846. Pergamon Press. Printed in England.

3-ACETYLALEURITOLIC ACID FROM THE SEEDS OF *PHYTOLACCA AMERICANA*

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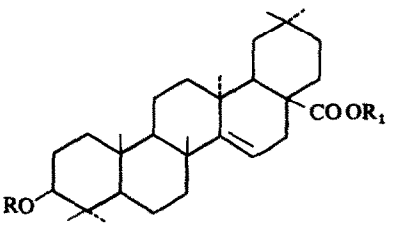
(*Received* 13 *May* 1977)

Key Word Index—*Phytolacca americana*; Phytolaccaceae; poke-weed; pentacyclic triterpenoid; acetylaleuritolic acid.

In the course of a continuing study of the terpenoids of *Phytolacca* growing in Korea [1], we isolated a triterpene acid from the seeds of *Phytolacca americana* L. The compound (**1a**), $C_{32}H_{50}O_4$, mp 301–302°, $[\alpha]_D^{25}$ + 23.1° (c = 0.6 in $CHCl_3$), λ_{max}^{EtOH} 204 nm (log ε, 3.84), gave a pink colouration in the Lieberman–Burchard test and showed in its IR spectrum acetoxyl peaks at 1735 and 1240 cm^{-1}, a carboxyl peak at 1689 cm^{-1} and trisubstituted double bond peak at 830 cm^{-1}. The compound (**1a**) afforded a monomethyl ester (**1b**), mp 238–239°, $[\alpha]_D^{25}$ + 19.38° (c = 1.07 in $CHCl_3$) by methylation with CH_2N_2, and gave a corresponding hydroxy acid (**1c**), mp 303–304°, $[\alpha]_D^{25}$ + 49.43° (c = 0.14 in $CHCl_3$) by hydrolysis with alkali. The methyl ester (**1b**) absorbed one equivalent of oxygen from perbenzoic acid, affording the corresponding epoxide (**2**), mp 200–201°, $[\alpha]_D^{25}$ + 42.85° (c = 0.323 in $CHCl_3$). This epoxide showed negative tetranitromethane reaction, thus indicating the presence of only one double bond in the compound (**1a**). The methyl ester (**1b**) showed in its NMR spectrum seven tertiary methyl signals between δ 0.84 to 0.94, an acetoxyl signal at 2.05 and a carbomethoxyl signal at 3.58, and two quartets centered at 5.50 (1 H, J = 4 and 8 Hz) indicating the presence of a trisubstituted double bond and at 4.46 (1 H, J = 7 and 9 Hz) due to a proton on a carbon atom bearing a secondary acetoxyl group. Thus this compound must be a monoacetoxypentacyclic triterpene monocarboxylic acid with a trisubstituted double bond, which does not belong to the β-amyrin series. Further more, the compound (**1a**), its methyl ester (**1b**) and the hydroxy acid (**1c**) were isomerized by HCl to 3-acetyloleanolic acid, methyl oleanolate acetate and oleanolic acid, respectively. Hence (**1a**) is a double bond isomer of acetyloleanolic acid.

The compound (**1a**) gave a brom-γ-lactone (**3**), mp 245–246°, IR 1767 cm^{-1}, under conditions adequate for the formation of this derivative from oleanolic acid, and was recovered unchanged after being heated at 305° for 5 min. Since (**1a**) did not show the ease of decarboxylation characteristic of β,γ-unsaturated acids, the double bond is in the γ,δ-position to the carboxyl group, i.e. it occupies the 14:15 position.

The mass spectra of (**1a**) and its methyl ester (**1b**) are in complete agreement with the assigned structure and typical fragmentation patterns expected for Δ^{14}-taraxerene derivatives (**2**). In both spectra, the peak arising



1a R = Ac R_1 = H
1b R = Ac R_1 = Me
1c R = H R_1 = H

out of retro-Diels–Alder fragmentation involving the 14:15 double bond in ring D is observed at *m/e* 344 which can then lead to the ion fragments appearing at *m/e* 329, 284 and 269 due to the loss of Me, MeCOOH, and both groups, respectively. The fragments formed by collapse of ring C are also observed. The spectrum of **1a** exhibits the fragment containing rings D and E and its further decomposition product at *m/e* 234 and 189, while that of **1b** at *m/e* 248 and 189, due to the alteration in the C-17 substituents.

On the basis of these results the terpenoid of mp 301° from the seeds of *P. americana* which was believed to be 3-acetyloleanolic acid by Burke and Le Quesne* [3] is now identified as 3-acetyltaraxer-12-en-28-oic acid (3-acetylaleuritolic acid) previously known only from *Aleurites montana* (Euphorbiaceae) [4]. It is identical with the triterpenoid isolated from the same plant seeds

Although the mps of (**1a**) and acetylaleuritolic acid reported by the authors (lit. [3], mp 278–281°) compared badly, a direct comparison (mmp, co-TLC and MS) with an authentic sample of methylaleuritolate acetate, kindly supplied by Dr. H. N. Khastgir, confirmed the identity of these two terpenoids. Moreover, myricadiol diacetate

*Neither acetyloleanolic acid nor oleanolic acid could be detected in any part of *P. americana* grown in Korea.

prepared from the compound (**1a**) by reduction with $LiAlH_4$ followed by acetylation was identical (mmp, co-TLC and MS) with the authentic sample [5], provided by Dr. (Miss) W.H. Hui.

Acknowledgement—The authors are grateful to Dr. Le Quesne for the authentic sample and to Deutscher Akademischer Austauschienst (DAAD) for a research fellowship to W.S.W.

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Phytochemistry, 1977, Vol. 16, pp 1846–1847. Pergamon Press. Printed in England.

SAPONINS AND TRITERPENES FROM *ILEX OPACA*

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(*Received* 22 *March* 1977)

Key Word Index—*Ilex opaca*; Aquifoliaceae; holly; saponins; triterpenes; oleanolic acid; ursolic acid; nonacosane.

INTRODUCTION

The presence of tannins and a possible glucoside in the leaves of *Ilex opaca* has been documented [1]. The dried and powdered emulsion layer resulting after shaking a leaf and berry infusion with $CHCl_3$ was reported to have the pharmacological properties of digitalis [2]. Several sugars have been identified in leaf extracts and inositol has been isolated from the unripened fruit [3, 4]. Phytochemical classes isolated or detected in other members of the genus *Ilex* include: polyphenols, saponins, triterpenes, sterols, and alkaloids [5].

RESULTS AND DISCUSSION

An examination of *I. opaca* for toxic principles revealed the presence of saponins in both leaf and fruit extracts. An EtOH leaf extract, prepared from material previously extracted with hexane and $CHCl_3$, was consistently lethal (death within 24 hr) to mice when injected intraperitoneally in doses of 500 mg/kg and hemolytic to human erythrocytes. Similar extracts from the ripe fruits were not toxic upon injection using doses of 1000 mg/kg, but EtOH extracts were hemolytic to red blood cells. Hemolytic indices [6] of both EtOH extracts were comparable to those obtained from identical concentrations of digitonin. Acidic hydrolysis of a portion of the toxic EtOH leaf extract after subsequent purification procedures [7] resulted in the isolation of 380 mg (0.072% yield) of oleanolic acid which was characterized as its acetate derivative (mp 263–264°, mmp 263–266°, ref. mp 264–266°, IR, NMR, MS). PLC (SGPF 254:$CHCl_3$–MeOH–H_2O, 13:6:2, lower phase) of another portion of the toxic EtOH leaf extract resulted in the isolation of a crude saponin (mp 126–133°) which was not toxic upon intraperitoneal injection at doses of 1000 mg/kg, but which was hemolytic to erythrocytes. TLC after acidic hydrolysis of this amorphous saponin demonstrated it to be a glucosidic mixture containing three unidentified aglycones.

A nontoxic (1000 mg/kg) and amorphous hexane extract of leaf material yielded 40 mg (0.12% yield) of mostly nonacosane (mp 63–64°, lit. mp 64°, IR, MS, 77.9% nonacosane as demonstrated with GLC) after column chromatography on Si gel and elution with $CHCl_3$–C_6H_6(1:1).

A purification [7] of a portion of a nontoxic (1000 mg/kg) Et_2O extract from ripe holly fruits resulted in the isolation of 185 mg (0.009% yield) of ursolic acid (mp 283–285°, mmp 282–284°, ref. mp 282–284°, IR, MS).

I. opaca has a long, confused, and, perhaps, unjustified reputation as a dangerous plant [8, 9]. However, teas prepared from holly leaves are reported to be beneficial and pleasant to the taste [10]. As examined by us, successive hexane, $CHCl_3$, EtOH, and H_2O extracts of the ripe fruits did not exhibit signs of acute toxicity when injected into mice and observed for 24 hr. A leaf toxin is present in EtOH extracts, but its characterization was not accomplished. However, the presence of saponins was demonstrated, and this could explain at least part of the toxicity of the leaf extracts. Potential harm from these compounds in humans is questionable [11]. The hemolysis of erythrocytes by EtOH extracts of holly leaves and fruits may be attributable to the saponin content; however, this activity is not usually associated with oral ingestion [12].

EXPERIMENTAL

Plants. Leaves and ripe fruits of American holly, *Ilex opaca* Ait. Authentic leaf material was obtained from the University

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