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ACIDIC TRITERPENOIDS FROM *LITHOCARPUS ATTENUATA*

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Key Word Index—*Lithocarpus attenuata*; Fagaceae; Triterpenoids: 3-methoxyoleana-2,12-dien-1-one, olean-12-en-1,3-dione, betulinic acid, oleanolic acid.

Abstract—Methylation of the acidic mixture (isolation through the sodium salt) obtained from the ether soluble part of an ethanol extract of the stems of *Lithocarpus attenuata* gave a mixture which on chromatography led to the isolation of 3-methoxyoleana-2,12-dien-1-one, olean-12-en-1,3-dione, betulinic acid and oleanolic acid. This is the first report of olean-12-en-1,3-dione as a natural product. 3-Methoxyoleana-2,12-dien-1-one was formed by partial methylation of olean-12-en-1,3-dione in the enol form.

INTRODUCTION

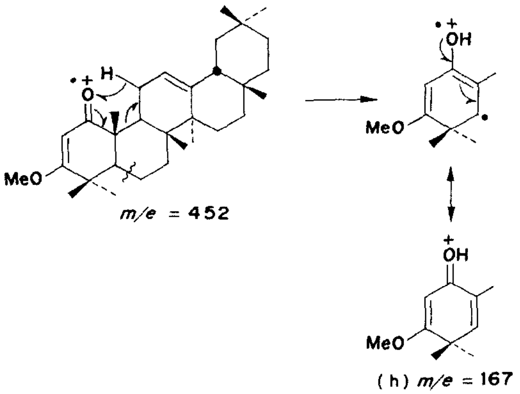
The light petroleum extracts of 10 *Lithocarpus* species (Fagaceae) have been examined in this laboratory for neutral triterpenoids and steroids [1]; the leaves of *L. attenuata* (Skan) Schky have been found to contain friedelin, friedelan-3β-ol, lupeol, taraxasterol, sitosterol, 22-hydroxyhopan-3-one, 3β,22-dihydroxyhopane and pachysandiol-*A*; and the stems friedelin, friedelan-3β-ol and sitosterol. The present work is an investigation of the subsequent ethanol extract of this plant for acidic triterpenoid constituents.

RESULTS AND DISCUSSION

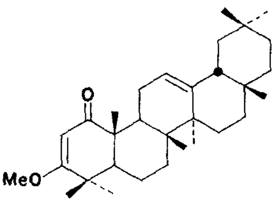
The ether soluble part of the ethanol extract of the stems was extracted with aqueous NaOH, and the precipitated "acid mixture" obtained by acidification of this alkaline solution was methylated with diazomethane and chromatographed on alumina. Elution with light petroleum first gave a compound (**1**), $C_{31}H_{48}O_2$ (M^+ 452), mp 235–236°, then another (**2**), $C_{30}H_{46}O_2$ (M^+ 438), mp ~120° resolidified, mp 205–208°, both of which gave positive Liebermann–Burchardt reaction and tetranitromethane test. Elution with light petrol–C_6H_6 (1:1) yielded methyl betulinate and methyl oleanolate.

The IR spectrum of (**1**) had υ_{max} 1675, 1615 (conjugated carbonyl) and 832 cm^{-1} (trisubstituted double bond), but showed no absorption at $\geqslant$ 1700 cm^{-1}, indicating the probable absence of carbonyl group of an ester. The conjugated double bond was also indicated by its UV spectrum: λ_{max} 251 nm (ϵ12000). Attempted hydrolysis with alkali led to the formation in low yield of a compound identical with (**2**), υ_{max} 3400, 1660, 1575 cm^{-1}; λ_{max} 285 nm (ϵ25000) shifted to 253 nm in dilute acid, indicative of the enol form of an α- or β-diketone. On being heated on the H_2O bath, it reverted to the diketo-form, mp 210–213° ν_{max} 1726, 1705 cm^{-1}. (**1**) Was possibly the enol methyl ether of (**2**), and this was confirmed by treating (**2**) with diazomethane, when the product was proved to be (**1**) with a small quantity of unchanged (**2**). From the above and the molecular formulae, (**1**) and (**2**) were pentacyclic triterpenoids. The NMR spectrum of (**1**) showed a vinyl $C\underline{H}_3O$ group [δ3·62 (3H, *s*)], one ordinary olefinic proton [δ5·25 (1H, *m*)], and another olefinic proton at δ5·03 (1H, *s*) representing the hydrogen on the double bond α to the enol ether group derived from the enol form of a β-dione (*A*) and not an α-dione (*B*) [2]. A β-dione system is only possible at C-1 and C-3 in ring *A* of common pentacyclic triterpenes. The spectrum also revealed eight tertiary methyl groups indicating the presence of an oleanane or rearranged oleanane skeleton.

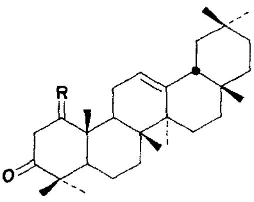
The unconjugated double bond was shown to be at C-12 by mass spectral fragments at *m/e* 233 (g), 218 (a), 205 (e), 203 (c), 189 (d) and 133 (f) [3]. Another strong peak at *m/e* 167 could be represented by species (h) if the carbonyl group was placed at C-1 and the methoxy at C-3. The mechanism for its formation is probably:



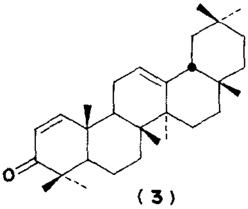
(**1**) Was thus 3-methoxyoleana-2,12-diene-1-one, and it followed that (**2**) was olean-12-en-1,3-dione. This was finally proved by reduction of (**1**) with $LiAlH_4$ followed by mild acid treatment to give the known 3-oxo-1,12-diene (**3**) [4], hydrogenation of which yielded β-amyrenone (**4**).



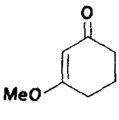
(**1**)



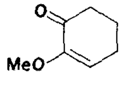
(**2**) R = O
(**4**) R = H_2



(**3**)



(A)



(B)

Based on the above structure, the NMR signals of the Me groups in (**1**) could be assigned as follows: δ0·82 (3H, *s*, C-28), 0·88 (6H, *s*, C-29, 30), 1·06 (3H, *s*, C-26), 1·18 (9H, *s*, C-23, 24, 27) and 1·23 (3H, *s*, C-26).

The natural occurrence of (**1**) and (**2**) have not been formerly reported, though Barton *et al.* [5] have isolated the two compounds as intermediates during their synthesis of β-amyrin. (**1**), of course, being non-acidic, was not a natural product itself. It was formed from (**2**) during the methylation of the "acidic" mixture. This is the second isolation of an oleanene derivative from plants with an oxo-group at C-1, the first being momordic acid (1-oxo-3β-hydroxyolean-12-en-28-oic acid) [6] from *Momordica cochinchinensis* (Cucurbitaceae). Also in pentacyclic triterpenoids the natural occurrence of 1,3-diones has only been reported once in the friedelane series from *Salacia prenoides* (Celastraceae) [7, 8]. The leaves were examined in the same way, but no acicic constituents could be isolated.

EXPERIMENTAL

IR spectra were recorded for KBr discs; NMR spectra in $CDCl_3$, UV spectra in 95% EtOH, and optical rotations in $CHCl_3$ solns. Light petrol had bp 60–80°. Known compounds were identified by TLC, mmp and IR spectral comparisons with authentic samples.

Extraction, isolation and methylation of "acidic" mixture. Air-dried stems (26 kg) after extractions with light petrol [1], were extracted 2× with 95% EtOH at room temp. for 1 week. The extract was vacuum dist to give a brown residue (450 g) which was repeatedly extracted with Et_2O. The ethereal soln was shaken with 1 M NaOH (×5). The alkaline extracts were acidified with 1 M H_2SO_4, and to the dried ppt. (10·0 g) obtained was repeatedly added CH_2N_2 in Et_2O. Removal of excess CH_2N_2 and ether gave a solid (10·5 g). Air-dried leaves (26 kg) were examined in the same way. No ppt was obtained on adding dil. H_2SO_4 to the alkaline extracts.

Isolation of products. The methylated mixture was dissolved in light petrol and chromatographed on alumina (300 g). Elution with light petrol gave a solid which was recrystallized from the same solvent to give prisms of 3-methoxyoleana-2,12-dien-1-one (**1**) (120 mg), mp 235–236°, $[\alpha]_D$ +223·5° (Lit. [5], mp 230–231°, $[\alpha]_D$ +229°) (found: M^+ 452, C, 82·7; H, 10·4. Calc. for $C_{31}H_{48}O_2$: M^+ 452, C, 82·25; H, 10·7%). Prolonged elution with light petrol yielded a crystalline mass which on repeated recrystallization from the same solvent afforded fine needles of olean-12-en-1,3-dione (**2**) (6·5 mg), mp 120–125°, resolidified, mp 205–208°, (Lit. [5], mps 116–120°, 198–201°) $[\alpha]_D$ +161·0° (found: M^+ 438. Calc. for $C_{30}H_{46}O_2$: M^+ 438). Elution with light petrol–C_6H_6 (1:1) gave methyl betulinate (50 mg) mp 228–230°, (from $CHCl_3$). ν_{max} 3550 (OH), 3080, 1650, 880 ($>C=CH_2$), and 1720, 1174 cm^{-1} (–COOMe). $C_{31}H_{50}O_3$. M^+ 470. Further elution with the same solvent yielded methyl oleanolate (5 mg), mp 198–201° (from C_6H_6), ν_{max} 3380 (OH), 3030, 1650, 850 ($>C=CH-$) and 1730, 1160 cm^{-1} (–COOMe). $C_{31}H_{50}O_3$. M^+ 470.

Attempted "hydrolysis" of (**1**). (**1**) (30 mg) was boiled under reflux in 10% KOH in MeOH (25 ml) for 4 hr. The product (20 mg) was purified by preparative TLC [C_6H_6–$CHCl_3$ (1:1)] to give fine needles (3 mg) mp 204–207° (after melting at *ca* 120° and resolidification) (from light petrol) $C_{30}H_{46}O_2$, M^+ 438, identical with (**2**).

Methylation of (**2**). (**2**) (5 mg) Was treated with excess CH_2N_2 in ether, the product (4 mg), showed 2 spots on preparative TLC [C_6H_6–$CHCl_3$ (1:1)]. The major component (3 mg) mp 234–236°, having higher R_f, was identical with (**1**), the minor component had same R_f as (**2**), but the quantity was too minute to be isolated.

Reduction of (**1**) *to olean*-1,12-*dien*-3-*one* (**3**). (**1**) (40 mg) In dry Et_2O (10 ml) containing $LiAlH_4$ (30 mg) was stirred at room temp. for 1 hr. The mixture was acidified with dil. HCl, and extracted with Et_2O [4]. The product from the ethereal soln was recrystallized from light petrol to give needles of olean-1,12-dien-3-one (**3**), (12 mg), mp 173–174°, $[\alpha]_D + 133°$ λ_{max} 229 nm (ϵ9850) [Lit. [9], mp 174–175°, $[\alpha]_D + 141°$ λ_{max} 230 nm (ϵ9700)]; ν_{max} 1680, 1620 (>C=C–C=O), 3050, 1650, 830 cm^{-1} (>C=CH–), $C_{30}H_{48}O$, M^+ 424.

Hydrogenation of (**3**) *to* β-*amyrenone* (**4**). (**3**) (8 mg) In ethanol (20 ml) was shaken with Adam's catalyst in H_2 for 2 hr. The product was recrystallized from light petrol to yield fine needles of β-amyrenone (5 mg), mp 178–181°, ν_{max} 1720 (>C=O), 1650, 820 cm^{-1}(>C=CH–).

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