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# Chemical Examination of Local Plants. Part XVI. Isolation of 9 α - Hydroxyursonic Acid from the Leaves of Egyptian *Eucalyptus rostrata.*

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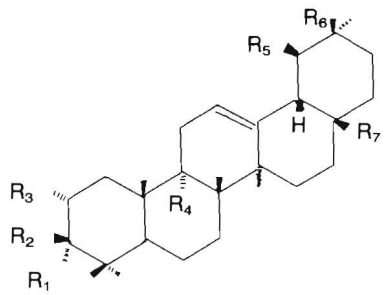
ABSTRACT. The ether extract of the defatted leaves of Egyptian *Eucalyptus rostrata*, yields a new triterpene; 9 α-hydroxyursonic acid together with ursolic acid and a series of normal and iso-paraffins.

The leaves of the plant *Eucalyptus rostrata* (Fam. Myrtaceae) are medicinally useful. The previous work described on the leaves of this plant concerns with the chemical examination of the volatile oils of commercial and medicinal importance.

We have previously investigated some species of the family Myrtaceae (El-Garby Younes 1975, Glen *et al.* 1967, Osman *et al.* 1974) and have reported the isolation of ursolic acid (I a), oleanolic acid (I b), maslinic acid (I c) and 2 α-hydroxyursolic acid (I d). However, no work has been reported on the leaves of Egyptian *Eucalyptus rostrata* concerning the isolation and identification of triterpenoids. This is the aim of the present studies, on the isolation and structural elucidation of 9 α-hydroxyursonic acid, a new triterpenic acid, together with ursolic acid.

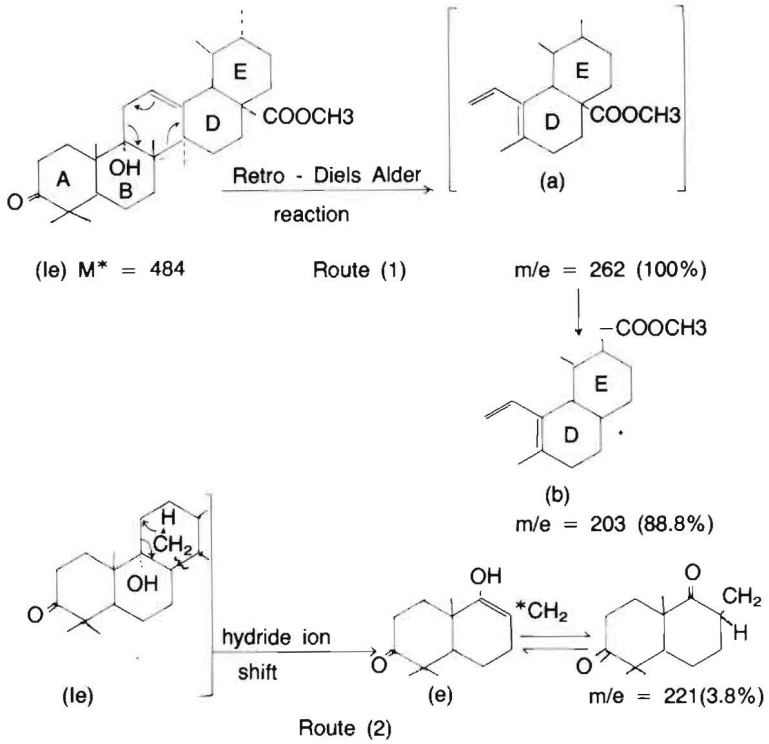
The defatted leaves were extracted with ether solvent and the neutral portion of the non-volatile fraction was analysed by GLC which showed the presence of; *n*-dodecane, *n*-triadecane, iso-triadecane, *n*-tetradecane, iso-tetradecane, *n*-pentadecane *n*-hexadecane, *n*-heptadecane, *n*-octadecane, *n*-nonadecane, *n*-eicosane, *n*-heneicosane, *n*-docosane, *n*-tricosane, *n*-tetracosane, *n*-pentacosane, *n*-hexacosane, *n*-heptacosane, *n*-octacosane, *n*-nonacosane, and *n*-triacontane. This series of hydrocarbons have not been previously isolated from the leaves of this plant.




a) $R_1 = R_3 = R_4 = R_6 = H$; $R_2 = OH$; $R_5 = CH_3$; $R_7 = COOH$
b) $R_1 = R_3 = R_4 = R_5 = H$; $R_2 = OH$; $R_6 = CH_3$; $R_7 = COOH$
c) $R_1 = R_4 = R_5 = H$; $R_2 = R_3 = OH$; $R_6 = CH_3$; $R_7 = COOH$
d) $R_1 = R_4 = R_6 = H$; $R_2 = R_3 = OH$; $R_5 = CH_3$; $R_7 = COOH$
e) $R_1 + R_2 = O$; $R_3 = R_6 = H$; $R_4 = OH$; $R_5 = CH_3$; $R_7 = COOCH_3$
f) $R_1 = R_3 = R_6 = H$; $R_5 = CH_3$; $R_2 = R_4 = OH$; $R_7 = CH_2OH$
g) $R_1 = R_3 = R_4 = R_6 = H$; $R_2 = OH$; $R_5 = CH_3$; $R_7 = COOCH_3$

The saponifiable portion of the ether extract contained two types of acids, a mixture of fatty acids and a mixture of triterpenic acids (TLC). The latter precipitated mixture was isolated from the main ether extract by filtration. This was methylated with diazomethane and chromatographed over a column of alumina. Elution with light petroleum yielded a mixture of two methyl esters (TLC), fractional crystallisation (light petroleum) of which gave the new triterpenic acid ester, methyl 9 α-hydroxyursonate (I e), the structure of which was based on the following evidence $\nu_{max}$ 3350, 1735 and 1720 $cm^{-1}$. (OH), ($COOCH_3$) and (> C=O), respectively. The compound gave a pink colour with Liebermann-Burchardt test for triterpenoids (Liebermann 1953) and +ve Zimmermann test (Zimmermann 1955) for 3-keto triterpenoids. The nuclear magnetic resonance spectrum of this compound showed a sharp singlet (3H) at τ, 6.37 (− $COOCH_3$) and an olefinic proton at τ, 4.5 (one H), thus, indicating the trisubstituted nature of the double bond. The molecular ion for this compound appeared at m/z 484, corresponding to molecular formula $C_{31}H_{48}O_4$. The most abundant ions appeared at m/z 262 (base peak) and at 203 (88,4%), suggesting that the compound was a pentacyclic triterpene with one double bond at $\Delta^{12(13)}$ and the carbomethoxyl group was located at $C_{17}$. This indicated that the molecule was either from the

ursane or oleanane series (Budzekiewicz *et al.* 1963 and Glen *et al.* 1967). The mass spectral fragmentation mechanism suggested two routes for the break down of this molecule. Route (1) occurred *via* retro-Diels Alder reaction to produce fragment ions (a) and (b) while route (2) occurred by hydride ion shift from the methyl at $C_{26}$ to give fragment ion (C) (Glen *et al.* 1967).




The position of the hydroxyl group was suggested to be in ring (A) or (B), from the mass spectral fragmentation of the compound. The carbon atoms that give tertiary hydroxyl groups in ring (A) or (B) are those at $C_5$ or $C_9$. The evidence for the hydroxyl at $C_9$, came from the dehydration of the compound with thionyl chloride and dry pyridine to give a conjugated homoannular diene; methyl 3-keto - $\triangle^{9(11),12(13)}$ ursadiene-28-oate. The ultraviolet spectrum of the diene showed an absorption band at 282 nm, while its infrared spectrum showed no peaks corresponding to hydroxyl group. The configuration of the substituents at $C_9$ in the ursane or oleanane series is always $\alpha$-configuration. The support for our compound

to belong to the ursane type, came from the study of its infrared spectrum in the regions $\nu_{max}$ 1350-1400 $cm^{-1}$(A) and $\nu_{max}$ 1240-1330 $cm^{-1}$(B). The ursane skeleton shows three peaks in region (A) and two peaks in region (B), (Snatzke *et al.* 1962), which are also present in the IR spectrum of our compound. Another evidence for the ursane skeleton for this compound came from the fact that this compound co-occurred with ursolic acid in the leaves of *Eucalyptus rostrata,* thus, suggesting that the compound could be related to ursolic acid. From the foregoing discussion, it was concluded that the structure of the compound was methyl 3-keto-9 α-hydroxyurus-12-ene-28-oate (I e).

Lithium aluminium hydride reduction of (I e) afforded the triol 9 α-hydroxyuvaol (I f). The IR spectrum of this compound showed no absorption in the carbonyl region. However, a strong absorption for a hydroxyl at $\nu_{max}$ 3350 $cm^{-1}$ supported the reduction of the carbonyl group.

Concentration of the mother liquour from which methyl 9 α-hydroxyursonate was isolated, afforded methyl ursolate (I g). Acetylation of (I g) with acetic anhydride-dry pyridine gave acetyl methyl ursolate, similar, in all respects, to an authentic specimen.

From the biogenetic point of view, we consider that ursolic acid is first biosynthesized in the leaves, followed by oxidation of the secondary hydroxyl group at position 3 and at position 9 from the α-face of the molecule to produce the tertiary α-hydroxyl group. 19-Hydroxyursolic acid, 19 α-hydroxyursonic acid (Brieskorn and Wunderer 1966) and 20 β-hydroxyursonic acid (Lawrie *et al.* 1967) all three metabolites of *Pyrus malus* and 2 α-hydroxyursolic acid (Glen *et al.* 1967) a metabolite of *Chamaenerion angustifolium* have previously been reported.

## Experimental

All melting points are uncorrected, IR spectra were recorded on an Acculab-9-spectrophotometer as KBr discs. Ultraviolet spectra were measured on a Beckmann model 25 spectrophotometer as ethanol solution, while NMR spectra were done as $CHCl_3$ solution on a 60 MHZ varian instrument and TMS was taken as internal standard. Mass spectra were determined on a Perkin Elmer HMU 6R spectrometer with ionisation energy 70 eV. Gas liquid chromatographic (GLC) analysis of the hydrocarbons was carried out on a Perkin Elmer Model 910 chromatograph, attached to flame ionisation detector with stainless steel column tube (10 ft × 3 mm) containing OV1 (15%) on chromosorb W (80-100 mesh), chart speed (5 mm/min), column temperature 330°C, carrier gas nitrogen flow rate 50 ml/min. Specific rotations were determined in chloroform solution using Pie-polarimeter model P 10. Light petroleum refers to the fraction of b.P. 60-80°. The phrase "in the usual manner" refers to the ether extraction followed by washing with water (or dilute acid or alkali where appropriate), then drying over anhydrous

sodium sulphate or calcium chloride, followed by filtration and evaporation of the ethereal solution *in vacuo.*

*Investigation of the leaves*

The dried and crushed leaves (3 Kg) were extracted in a Soxhlet apparatus for 72 hr with light petroleum. The defatted leaves were extracted with ether solvent for another 72 hr. The latter extract was concentrated under vacuum to yield a green solid (280.02) after filtration. Evaporation of the ether from the filtrate gave a dark green viscous oil (300 g). This was steam distilled to furnish a yellow volatile oil (2.37 g), and a non-steam volatile fraction (189.7 g). The latter was hydrolyzed with ethanolic KOH (10., 1900 ml) for 6 hr over a water bath. Removal of most of the alcohol followed by dilution with excess water and extraction with ether solvent afforded a yellow brown gum (123.5g), after concentration of the extract *in vacuo.* A column chromatography of this gum (2.0 g) over alumina (50 g) and elution with light petroleum gave a pale yellow wax (0.35g) which was analysed by GLC. The results of this analysis are shown in the following table :

GLC analysis of the paraffins of the neutral fraction from the ether extract of the leaves of *Eucalyptus rostrata*

| No. | Name of paraffine | Retention time ($t_R$) | Relative retention time | percentage (%) |
|---|---|---|---|---|
| 1 | *n*-Dodecane | 3.06 | 0.164 | 5.58 |
| 2 | *n*-Triadecane | 3.74 | 0.200 | 11.20 |
| 3 | iso-Triadecane | 4.30 | 0.231 | 00.22 |
| 4 | *n*-Tetradecane | 4.80 | 0.257 | 1.05 |
| 5 | iso-Tetradecane | 5.61 | 0.301 | 0.05 |
| 6 | *n*-Pentadecane | 6.16 | 0.331 | 4.67 |
| 7 | *n*-Hexadecane | 8.20 | 0.440 | 0.78 |
| 8 | *n*-Heptadecane | 11.09 | 0.595 | 33.62 |
| 9 | *n*-Octadecane | 13.48 | 0.724 | 0.05 |
| 10 | *n*-Nonadecane | 15.92 | 0.855 | 0.01 |
| 11 | *n*-Eicosane | 18.61 | 1.000 | 0.19 |
| 12 | *n*-Heneicosane | 24.07 | 1.293 | 0.35 |
| 13 | *n*-Docosane | 28.27 | 1.519 | 0.24 |
| 14 | *n*-Triacosane | 23.80 | 1.723 | 14.22 |
| 15 | *n*-Tetracosane | 39.51 | 2.123 | 0.13 |
| 16 | *n*-Pentacosane | 44.59 | 2.396 | 0.24 |
| 17 | *n*-Hexacosane | 49.50 | 2.659 | 0.25 |
| 18 | *n*-Heptacosane | 52.91 | 2.843 | 0.55 |
| 19 | *n*-Octacosane | 58.88 | 3.763 | 6.53 |
| 20 | *n*-Nonacosane | 64.56 | 3.469 | 11.00 |
| 21 | *n*-Triacontane | 70.17 | 3.770 | 1.65 |

Continuous elution of the column using light petroleum, and then ether, gave a yellow liquid (0.26 g), which was left aside without further investigation.

The solid (280.0 g) isolated from the main ether extract showed absorption at $\nu_{max}$ 1690 and 3350 $cm^{-1}$ typical of (COOH). It gave a positive Liebermann Burchardt test for triterpenoids. The crude solid was methylated with ethereal diazomethane and the reaction mixture showed four spots on a TLC plate (benzene-ether; 1:4 v/v). The mixture was chromatographed over an alumina (250 g) column. Elution of the column with light petroleum and then with ether gave a mixture of two spots $R_f$ (0.4) and (0.45) (light petroleum - ether 3:1 v/v). Fractional crystallisation of this mixture from light petroleum gave a substance which was recrystallised three times from MeOH-$CHCl_3$ to give colourless needles of methyl 3-keto-9α-hydroxyurs -12-en-28-oate (Ie; 0.26g) yield (2.6%), m.p. 181°, $[\alpha]_D$ + 40.19 (C, 0.64), $\nu_{max}$ 1720 $cm^{-1}$ (> C=O), 3360 $cm^{-1}$ (OH). It gave a positive Liebermann-Burchardt test for triterpenoids and positive Zimmermann test for 3-keto triterpenes. The NMR spectrum showed signals at τ, 6.37 ($COOCH_3$), τ, 4.5 (one olefinic hydrogen). UV spectrum showed band at $\lambda_{max}^{EtOH}$ 210 nm. The molecular weight by mass spectrum is 484 corresponding to molecular formula $C_{31}H_{48}O_4$. (Found: C, 77.09; H, 9.9 $C_{31}H_{48}O_4$. requires 76.80; H,9.9 %).

Reduction of methyl 3 keto -9-α-hydroxyurs-12-en-28-oate(Ie), with lithium aluminium hydride: Methyl 9 α-hydroxyursonate(Ie; 0.59.) in dry ether (50 ml) was refluxed with a suspension of LAH (0.5 g) in dry ether (50 ml) over a water bath for 3 hr.

The reaction mixture was cooled and acidified with dilute sulphuric acid, then extracted with ether. Working up as usual, gave leaflet crystals ($CH_3OH$; 0.35 g) of 9 α-hydroxyuvaol (If). The IR spectrum shows strong band at $\nu_{max}$ 3350 $cm^{-1}$ (OH) and no bands corresponding to carbonyl groups are detected, m.p. 196°, $[\alpha]_D$ + 11.30° (C, 0.63). (Found: C, 78.28; H, 11.01. $C_{30}H_{50}O_3$ requires C, 78.60; H, 10.9%).

*Dehydration of Methyl 9 α-hydroxyursonate (I e) with Thionyl Chloride and Dry Pyridine*

Methyl 9-α-hydroxyursonate (Ie; 0.20 g) was treated with thionyl chloride-dry pyridine mixture (1:8 v/v; 1 ml) at O° for 15 min, then poured onto ice and extracted with ether. Working up on the reaction mixture as usual gave colourless needles of methyl 3 keto-$\Delta^{9(11),12(13)}$ ursadiene -28-oate (0.14 g) (MeOH-$CHCl_3$), m.p. 103−4°, $[\alpha]_D$ + 44.7 (C, 0.89), $\lambda_{max}^{EtOH}$ 282nm. (Found: C, 77.32; H, 9.3. $C_{31}H_{46}O_4$ requires C, 77.1; H,9.5%). The IR spectrum showed no absorption bands corresponding to hydroxyl groups.

*Separation of Methyl Ursolate (I g)*

Concentration of the mother liquour from which methyl 9 α-hydroxyursonate

(Ie) was isolated, it gave colourless resettes (MeOH-$CHCl_3$), showing a single spot on a TLC, $R_f$ value 0.45 (light petroleum; ether 3:1 v/v), identical in all respects with authentic sample of methyl ursolate m.p. and m.m.p. 169-70°, $[\alpha]_D$ + 59 (C, 0.79).

Acetylation of methyl ursolate (1.0 g) with acetic anhydride (3 ml) and dry pyridine (7.5 ml) over a water bath for 2 hr followed by extraction with ether of the cold reaction mixture and working up the reaction mixture as usual, yielded methyl acetyl ursolate, identical in all respects with that of authentic sample, IR, m.p. and m.m.p. 239°, $[\alpha]_D$ + 54.0 (C, 0.71). The NMR spectrum showed signals at τ, 7.89 ($-O-CO-C\underline{H}_3$) and at τ, 6.35 ($-COO\ C\underline{H}_3$).

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# الملخص الكيميائي للنباتات المحلية ـ الجـزء السادس عشر
# فـصـل ٩ ألـفـا ـ هـيـدروكسي حمض الأورسونيك من أوراق نبات الكافور المصري

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الزقازيق ـ جمهورية مصر العربية

لقد فصل من مستخلص الإثير لأوراق نبات الكافور المصري تربين ثـلاثي جديـد أطلق عليه ـ ٩ ألفـا ـ هيدروكسي حمض الأورسونيك، هذا بالإضافة إلى حمض الأورسـوليك وكـذلك سلسلة من البرافينات العادية والأيزوبرافينـات أمكن التعرف عليها بواسطة كروماتوجرافيا الغاز.