

## ROXBURGHONIC ACID—A FRIEDELANE TRITERPENOID KETO ACID OF THE LEAF OF *PUTRANJIVA ROXBURGHII*

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**Abstract**—The acidic fraction of the alcoholic extract of the leaf of *P. roxburghii* Wall., yielded a new triterpene acid, roxburghonic acid, which has been shown by physico-chemical methods to be 3-keto-friedelan-25-oic acid.

RECENTLY a number of triterpenoids have been reported<sup>1-6</sup> from the bark and the leaf of *Putranjiva roxburghii* Wall. (Euphorbiaceae). While working with the leaf constituents, we have now isolated a new crystalline triterpenoid acid, m.p. > 340°, named roxburghonic acid. Apart from the acid, the ether-soluble acidic fraction of the leaf extractive (alcoholic) also yielded a mixture of biflavonoids<sup>7</sup> on chromatography.

Roxburghonic acid (I), C<sub>30</sub>H<sub>48</sub>O<sub>3</sub>, showed positive Liebermann-Burchard (pink colour) and negative tetranitromethane tests indicating it to be a saturated triterpenoid which was further confirmed by the absence of any olefinic proton in the NMR spectra of its methyl ester. The i.r. spectra of roxburghonic acid showed absorption at 1700 and 1720 cm<sup>-1</sup> corresponding to a six membered cyclic ketone and a carboxylic group respectively. It did not show the presence of any hydroxyl absorption and is therefore a keto-acid. The physico-chemical characteristics of roxburghonic acid indicated it to be belonging to friedelane series, as do the other triterpenoids of *P. roxburghii*.<sup>1-6</sup>

On methylation with diazomethane roxburghonic acid (I) yielded a new monomethyl ester, methyl roxburghonate (II), C<sub>31</sub>H<sub>50</sub>O<sub>3</sub> (M<sup>+</sup> 470), m.p. 173–74°. The i.r. spectrum of II showed absorption at 1710 and 1725 cm<sup>-1</sup> for the cyclohexanone and the ester-carbonyl respectively. The NMR spectrum of roxburghonate (II) in CDCl<sub>3</sub> showed the presence of six tertiary methyl groups between δ 0.90–1.55 ppm and a secondary methyl at δ 0.85 (*J* = 6–7 Hz) ppm and a carbomethoxy methyl at δ 3.59 ppm. The presence of protons *alpha* to the cyclohexanone was observed as broad signals at δ 2.1 and δ 2.25 ppm corresponding to 3 protons.

The ORD spectrum of the methyl roxburghonate (II) was found to be identical to that of friedelin<sup>8</sup> (Fig. 1) showing negative Cotton effect having peak at (α)<sub>267</sub> + 128° and trough

<sup>1</sup> P. SEN GUPTA, A. K. CHAKRABORTY, A. M. DUFFIELD, L. J. DURHAM and C. DJERASSI, *Tetrahedron* **24**, 1205 (1968).

<sup>2</sup> H. S. GARG and C. R. MITRA, *Phytochem.* **7**, 2053 (1968).

<sup>3</sup> G. R. CHOPRA, A. C. JAIN and T. R. SESHADRI, *Current Sci.* **37**, 301 (1968).

<sup>4</sup> H. S. GARG and C. R. MITRA, *Tetrahedron Letters* 231 (1969).

<sup>5</sup> P. SEN GUPTA and J. MUKHERJEE, *Tetrahedron* **24**, 6259 (1968).

<sup>6</sup> H. S. GARG and C. R. MITRA, *Planta Med.* in press.

<sup>7</sup> H. S. GARG and C. R. MITRA, *Tetrahedron* in press.

<sup>8</sup> C. DJERASSI, R. RINIKER and B. RINIKER, *J. Am. Chem. Soc.* **78**, 6362 (1956).

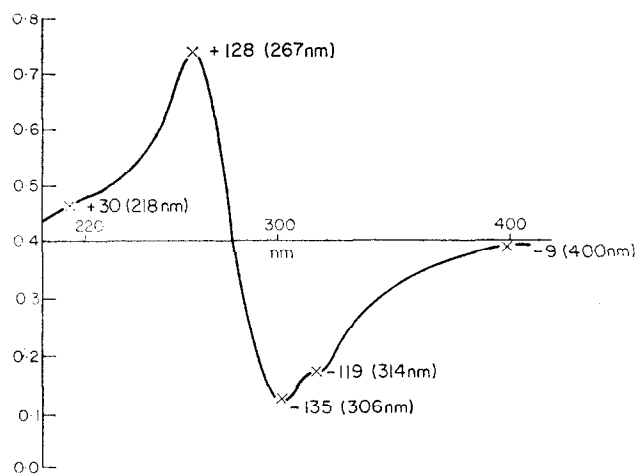
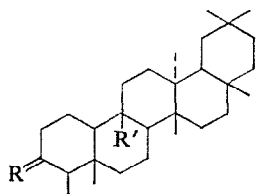
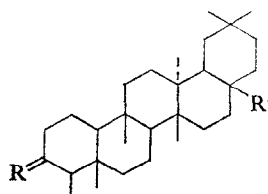


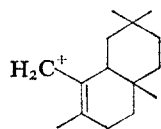
FIG. 1. ORD CURVE OF METHYL ROXBURGHONATE IN METHANOL.



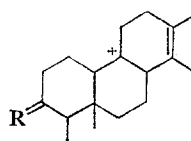
- I R = O, R' = COOH  
 II R = O, R' = COOCH<sub>3</sub>  
 III R = H<sub>2</sub>, R' = COOCH<sub>3</sub>  
 VI R = O, R' = CH<sub>2</sub>OH  
 VII R = O, R' = CHO



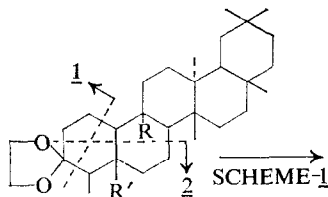
- V R = O, R' = COOCH<sub>3</sub>  
 Va R =  $\begin{matrix} \text{OH} \\ \diagup \end{matrix}$ , R' = COOH  
            $\diagdown$   
           H



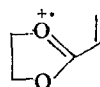
'a'  
 (m/e 205)



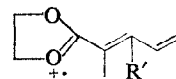
3  
 R = O, m/e 259  
 R = H<sub>2</sub>, m/e 245



IV R = COOCH<sub>3</sub>, R' = CH<sub>3</sub>



1  
 m/e 99



2  
 R' = CH<sub>3</sub>, m/e 153  
 R' = COOCH<sub>3</sub>, m/e 197

at  $(\alpha)_{306}-135^\circ$  with a secondary trough at  $(\alpha)_{314}-119^\circ$ , a characteristic of the friedelane 3-ketones.<sup>8</sup> This suggested roxburghonic acid (I) to be a friedelin carboxylic acid.

Methyl roxburghonate, on Clemmensen reduction as well as Huang-Minlon reduction, followed by methylation, yielded a new desoxy-ester (III),  $C_{33}H_{52}O_2$  ( $M^+$  456), m.p. 168–70°. The i.r. spectrum of the desoxy ester (III) showed absorption for the ester carbonyl only at  $1725\text{ cm}^{-1}$  and disappearance of the cyclic ketone. The NMR spectrum of III also showed the disappearance of the protons *alpha* to the carbonyl group between  $\delta$  2–3 ppm. Thus the carboxyl group of roxburghonic acid did not have any proton in the *alpha* position and must be present in tertiary position.

The easy reduction of the cyclic ketone of methyl roxburghonate as well as its ORD confirmed the presence of C-3 ketone in roxburghonic acid (I). This was further supported by easy formation of ethylene ketal in presence of *p*-toluene sulphonic acid, yielding the 3-ethylene ketal of methyl roxburghonate (IV),  $C_{33}H_{52}O_4$  ( $M^+$  514) m.p. 196–198°. The NMR spectrum showed a quartet-like signal centred at  $\delta$  3.88–3.92 (4H) ppm for the ketal protons in addition to other signals.

The carbomethoxy group was found to be hindered, since the desoxy-ester (III) could not be reduced to the corresponding alcohol by lithium aluminium hydride. Thus the possibility of the carboxyl group being at C-17, the common site of the carboxyl in triterpene, was ruled out. Further, methyl-roxburghonate was found to be different from the keto-ester (V) obtained from canophyllic acid (Va),<sup>9</sup> the only known carboxylic acid in the friedelane series.

Mass spectral studies with II, III, IV revealed the presence of the keto group as well as the carbomethoxy group in rings A-B-C. The important mass peaks of these derivatives are listed in Table 1. The ( $M^+-59$ ) peak in all the three derivatives showed the loss of the carbomethoxyl radical from the molecules. The abundant  $m/e$  205 peak in all the compounds, corresponds to the fragment  $a^{1,10}$  constituting rings *D* and *E* having no new substituents groups present therein. This rules out the possibility of the carboxyl as C-26, C-27, C-28, C-29 or C-30. Thus the carboxyl group could either be placed at C-24 or C-25 (the C-23 position being ruled out on the basis of NMR).

TABLE 1

Compound	Mass fragments ( $m/e$ )								
	$M^+$	$M^+-59$	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	1	2	3
Methyl roxburghonate (II)	470	411	205	346	331(w)	317	—	—	259
Desoxy roxburghonate (III)	456	397	205	332	317(w)	303	—	—	245
3-ketal roxburghonate (IV)	514	455	205	390(w)	—	—	99	153	—

In order to distinguish between C-24 and C-25 positions for the carboxyl group, the mass spectral studies with the ethyleneketal-ester (IV) were of great help. It has been established that C-3 ketals give rise to specific fragmentation<sup>11</sup> of ring *A* and *B* along routes

<sup>9</sup> T. R. GOVINDACHARI, N. VISWANATHAN, B. R. PAI, U. RAMDAS RAO and M. SRINIVASAN, *Tetrahedron* **23**, 1901 (1967).

<sup>10</sup> (a) J. L. COURTNEY and J. S. SHANNON, *Tetrahedron Letters* **13** (1963);

(b) J. S. SHANNON, C. G. MACDONALD and J. L. COURTNEY, *Tetrahedron Letters* **173** (1963);

(c) H. BUDZIKIEWICZ, J. M. WILSON and C. DJERASSI, *J. Am. Chem. Soc.* **85**, 3688 (1963).

<sup>11</sup> H. BUDZIKIEWICZ, C. DJERASSI and D. H. WILLIAMS, *Mass Spectrometry of Organic Compounds*, p. 266, Holden Day, San Francisco (1967).

1 and 2 (Scheme-I). In the present case the corresponding fragment for route 1 would be observed at  $m/e$  99 and for 2 at  $m/e$  197 or  $m/e$  153 with or without C-24 carbomethoxyl substituent. However, in the present case the peaks are observed at  $m/e$  99 and  $m/e$  153 so that C-24 does not carry the carboxyl group. Thus, it may safely be placed at C-25, which is in agreement with its hindred nature. Courtney *et al.*<sup>10b</sup> have isolated C-25-ol (VI) and C-25-al (VII) derivatives of friedelin from *Siphonodon australe* Benth. and showed that they could not be oxidized to the corresponding acid. Similarly the reduction of the ester to the alcohol must also be hindred since the carboxyl at C-25 would be subject to 1:3 interactions by two axial methyl groups attached at C-5 and C-14.

The other mass spectral features corresponding to fragments *b*, *c* and *d* were similar to those reported in case of putranjivadiene,<sup>1</sup> and other friedelin series of compounds.<sup>10</sup> The fragment at  $m/e$  259 in II and  $m/e$  245 in III are assigned to fragment 3 as reported in the case of friedelin -25-al and 3-keto-friedelan-25-al.<sup>10b</sup>

Thus roxburghonic acid is 3-keto-friedelan-25-oic acid (I) and becomes the second example of an acid in this series, the first being the hydroxy-acid, canophyllic acid (Va). From the biogenetic point of view, the existence of a C-25 carboxylic acid in nature is of interest as it could not be obtained in the laboratory by oxidation of the corresponding aldehyde.<sup>10b</sup>

## EXPERIMENTAL

M.ps were taken in open capillaries and are uncorrected. The i.r. spectra were recorded in KBr films and NMR spectra were run in  $\text{CDCl}_3$  using TMS as internal reference.

### Isolation of Roxburghonic Acid

The air dried leaves (5 kg) were extracted with alcohol (80–85%) ( $5 \times 5$  l.) by percolation at room temp. The dark greenish brown alcoholic extract was exhaustively extracted with ether ( $4 \times 1$  l.) in the cold ( $5^\circ$ ) and the ethereal solution thus obtained was separated into acidic and neutral fractions by washing with 0.5% NaOH solution. The alkali soluble fraction on acidification and extraction with ether deposited a microcrystalline residue (1.5 g) on concentration. This residue, on chromatography over silica gel, eluting with  $\text{CHCl}_3$ , yielded a fraction showing a positive Liebermann–Burchard test (350 mg) which on recrystallization (alcohol/ $\text{CHCl}_3$ ) yielded fine needles of roxburghonic acid,  $\text{C}_{30}\text{H}_{48}\text{O}_3$  m.p.  $> 340^\circ$ . (Found: C, 77.97; H, 10.76;  $\text{C}_{30}\text{H}_{48}\text{O}_3$  requires: C, 78.94; H, 10.76%.)

### Methyl Roxburghonate (II)

250 mg of roxburghonic acid in methanol (10 ml) was methylated with excess ethereal  $\text{CH}_3\text{N}_2$ . The product was crystallized from dilute alcohol (80%) to yield silky needles (250 mg) of methyl roxburghonate m.p.  $173\text{--}74^\circ$ . NMR:  $\delta$  0.70, 0.82, 0.90, 0.95, 0.99, 1.05 ( $7 \times \text{CH}_3$ ), 1.16, 1.3, 1.42, 1.51, 2.1 and 2.25 (3H) and 3.59 ( $\text{COOCH}_3$ ) ppm. Mass: ( $\text{M}^+$ ) 470, 455, 411, 410, 396, 346, 333 (W), 318, 317(s), 291, 277, 259, 231, 217, 205 (base ion peak), 179, 123 etc.  $m/e$  ORD: ( $\alpha$ )<sub>218</sub>  $+ 30^\circ$  ( $\alpha$ )<sub>267</sub>  $+ 128^\circ$ , ( $\alpha$ )<sub>306</sub>  $-135^\circ$ , ( $\alpha$ )<sub>314</sub>  $-119^\circ$ , ( $\alpha$ )<sub>400</sub>  $-9^\circ$  (Found: C, 78.33; H, 10.81;  $\text{C}_{31}\text{H}_{50}\text{O}_3$  requires: C, 79.14; H, 10.64%.)

### Huang Minlon Reduction of Methyl Roxburghonate to Desoxyroxburghonate (III)

A mixture of methyl roxburghonate (100 mg), NaOH (50 mg) ethylene glycol (2 ml) and  $\text{N}_2\text{H}_4$ ,  $\text{H}_2\text{O}$  (0.5 ml) was refluxed on oil bath at  $170\text{--}180^\circ$  for 2 hr, when the condensor was removed until the temperature of the solution had reached  $195\text{--}200^\circ$ , and refluxing was continued for another 2 hr. The mixture was cooled, acidified and extracted with ether. The residue from ether extract was treated with freshly prepared ethereal  $\text{CH}_3\text{N}_2$  in the cold. On crystallization with ethanol the product yielded fine needles of desoxyroxburghonate (III), m.p.  $168\text{--}170^\circ$ . NMR:  $\delta$  0.74, 0.92, 1.0, 1.03 ( $7 \times \text{CH}_3$ ), 1.18, 1.20, 1.26, 1.4 and 3.59 ( $\text{COOCH}_3$ ) ppm. Mass: 456 ( $\text{M}^+$ ), 441, 425 (W), 397, 382, 332, 331, 303, 277, 245, 217, 205, 203, 191, 179, etc.  $m/e$ . (Found: C, 80.70; H, 11.38;  $\text{C}_{31}\text{H}_{52}\text{O}_2$  requires: C, 81.5; H, 11.43%.)

### Clemmensen Reduction of Methyl-roxburghonate to III

Methyl roxburghonate (5 mg) in alcohol (10 ml) was refluxed with freshly prepared Zn–Hg in presence of HCl (10N) for 3 hr. The product on crystallisation (alc) yielded needles of III as above, m.p. and mixed m.p.  $168\text{--}170^\circ$  (i.r. superposable).

*Reduction of Desoxy-roxburghonate*

100 mg of desoxy-roxburghonate was stirred with  $\text{LiAlH}_4$  (200 mg) in tetrahydrofuran (10 ml) for 2 hr and then refluxed on a water bath. The product was recrystallized (alc) to yield fine needles of unreacted desoxy-roxburghonate, m.p. and mixed m.p. 168–170° (i.r. superposable to III).

*3-Ethylene Ketal of Methyl Roxburghonate (IV)*

100 mg of II was taken up in 5 ml of benzene and refluxed with 2.5 ml of ethylene glycol and 25 mg of *p*-toluene sulphonic acid for 6 hr using a Dean–Stark water separator, concentrated and extracted with ether, washed free from acid and the residue was recrystallized (alc) to yield needles of the ethylene ketal (IV) m.p. 196–198°, NMR:  $\delta$  0.70, 0.83, 0.99, 1.0, 1.05, 1.12, ( $7 \times \text{CH}_3$ ), 1.25, 1.55, 3.59, ( $\text{COOCH}_3$ ), 3.88, 3.92 ppm. Mass: 514 ( $\text{M}^+$ ) (base peak), 499, 485, 455, 346, 291, 279, 231, 217, 205, 167, 153, 123, and 99 etc. *m/e*. (Found: C, 77.55; H, 11.28;  $\text{C}_{33}\text{H}_{54}\text{O}_4$  required: C, 77.04; H, 10.50%.)

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