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# 1,3-Dimethyl-2-oxabicyclo[2,2,2]-octane-3-methanol and 1,3-Dimethyl-2-oxa-bicyclo[2,2,2]octane-3-carboxylic Acid, Urinary Metabolites of 1,8-Cineole

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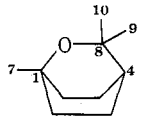
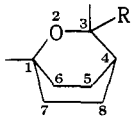
*Abstract*

Examination of urinary metabolites from brushtail possums (*Trichosurus vulpecula*) maintained on a diet of fruit impregnated with 1,8-cineole (1) yielded two new compounds shown by spectral analysis, synthesis and interconversion to be 1,3-dimethyl-2-oxabicyclo[2,2,2]octane-3-methanol (2) (9-hydroxycineole) and 1,3-dimethyl-2-oxabicyclo[2,2,2]octane-3-carboxylic acid (3) (cineol-9-oic acid).

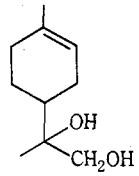
Previous attempts to isolate urinary metabolites of 1,8-cineole (1) have met with limited success: no metabolites were isolated from sheep,[1] glucuronides of 2- or 3-hydroxycineole were suspected from rabbits[2] and a crystalline mixture of unidentified hydroxycineoles was reported from possums.[3] We now report a re-examination of the urine of cineole-fed possums which resulted in the isolation of *p*-cresol, and the previously undescribed 1,3-dimethyl-2-oxabicyclo[2,2,2]octane-3-methanol (2) (9-hydroxycineole) and 1,3-dimethyl-2-oxabicyclo[2,2,2]octane-3-carboxylic acid (3) (cineol-9-oic acid). Benzoic acid, a fourth isolate, was found to be a metabolite of the ingested fruit.




(1)




| | R |
|---|---|
| (2) | $CH_2OH$ |
| (3) | $CO_2H$ |
| (5) | $CO_2Me$ |




(4)

The ethyl acetate extract of the urine, after washing with aqueous sodium bicarbonate, sodium carbonate and sodium hydroxide yielded a neutral crystalline metabolite $C_{10}H_{18}O_2$ subliming at 63–92° (m.p. 99°). Mass spectra of the metabolite and its α-naphthylurethane (m.p. 117°) also indicated this formula and the presence of a primary alcohol group. Proton magnetic resonance showed signals at $\delta$ 1·06 (methyl), 1·27 (methyl), 2·05 (hydroxyl) and 3·51 (oxymethylene) which shifted with $Eu(fod)_3$ by 7, 13, 76 and 22 ppm per molar equivalent respectively. Consequently, the methanol

[1] Wright, S. W., *Univ. Queensl. Pap., Dep. Chem.*, 1945, **1**(25), 1.
[2] Hämäläinen, J., *Skand. Arch. Physiol.*, 1910, **24**, 1.
[3] Cleland, J. B., M.Sc. Thesis, University of Adelaide, 1946.

group is positioned at C 3 and the metabolite assigned as 1,3-dimethyl-2-oxabicyclo-[2,2,2]octane-3-methanol (2) (9-hydroxycineole). This structure was confirmed by synthesizing (2) from limonene by oxidation to uroterpenol (4) with lead tetraacetate[4] and subsequent cyclization by the mercuric acetate/sodium borohydride etherification method which Coxon *et al.* had successfully applied to the synthesis of cineole (1) from α-terpineol.[5]

The reacidified sodium hydroxide washings of the extract yielded *p*-cresol.

Two methyl esters were isolated from the methylated acidic fraction and purified by alumina chromatography. The less-polar ester was identical to methyl benzoate and the second component had a $C_{11}H_{18}O_3$ formula. The latter was shown by proton magnetic resonance spectroscopy and lithium aluminium hydride reduction to 1,3-dimethyl-2-oxabicyclo[2,2,2]octane-3-methanol (2) to be methyl 1,3-dimethyl-2-oxabicyclo[2,2,2]octane-3-carboxylate (5). The isolation of benzoic acid from the urine of a control possum indicated that only *p*-cresol, (2) and (5) were genuine metabolites of 1,8-cineole.

This is, to our knowledge, the first successful structural elucidation of cineole metabolites from any animal species. The diffuse melting point reported for the hydroxycineole metabolites which Cleland isolated from possum urine[3] suggests that she had actually isolated 1,3-dimethyl-2-oxabicyclo[2,2,2]octane-3-methanol (2).

## Experimental

*General*

Analyses were carried out by the Australian Microanalytical Service, Melbourne. Melting points were determined on a Reichert hot-stage apparatus and are uncorrected. Infrared spectra were measured on neat films and Nujol mulls for liquids and solids respectively on a Unicam SP 1200G spectrophotometer. Proton magnetic resonance spectra were measured on a Varian HA 100 instrument. Chemical shifts ($\delta$) are expressed in ppm from internal reference tetramethylsilane and europium shift values (ΔEu) in ppm per molar equivalent. Mass spectra were obtained at 70 eV from an AEI MS902 spectrometer. Analytical gas chromatography was carried out on a Perkin–Elmer 900 gas chromatograph with a 50 ft by 0·02 in. internal diameter support coated open tubular column containing FFAP as a stationary phase and with helium as a carrier gas. Gas chromatography percentages were determined with a Hewlett–Packard 3370 A electronic integrator. Light petroleum had a boiling point 40–60°. Alumina 'Woelm neutral TLC' was used for thin-layer chromatography and Bio-Scientific aluminium oxide (neutral) for preparative chromatography.

*Isolation of Metabolites*

The brushtail possum, *Trichosurus vulpecula*, was maintained for 8 days on a diet of fruit which had been impregnated with cineole (28·8 g) and the urine (2 l.) collected and extracted with ethyl acetate (5 × 200 ml). The extract was washed sequentially with 5% $NaHCO_3$ (3 × 100 ml), 10% $Na_2CO_3$ (3 × 100 ml) and 5% NaOH (3 × 100 ml) solutions leaving a neutral extract (0·43 g) which solidified on cooling. Recrystallization from hexane yielded gas-chromatographically pure needles of *1,3-dimethyl-2-oxabicyclo[2,2,2]octane-3-methanol* (2), sublimed 63–92°, m.p. 99° (Found: C, 70·7; H, 10·5; O, 18·5. $C_{10}H_{18}O_2$ requires C, 70·5; H, 10·6; O, 18·8%). $\nu_{max}$ 3230, 1300, 1270, 1208, 1040, 980, 850 $cm^{-1}$; $\delta$ ($CDCl_3$) 1·06, s, ΔEu 7, 1-Me; 1·27, s, ΔEu 13, 3-Me; 2·05, temp. var., ΔEu 76, OH; 3·40, dd, *J* 7·4, 10·6 Hz, ΔEu 20, $CH_AOH$; 3·62, dd, *J* 3·5, 10·6 Hz, ΔEu 23, $CH_BOH$; *m/e* 170 (0·2%), 140 (5), 139 (51), 95 (24), 43 (100). *α-Naphthylurethane*, m.p. 117°; $\nu_{max}$ 3280, 1700, 1640, 1600, 1560, 1510, 1415, 1350, 1255, 1225, 1205, 1105, 1065, 1010, 790, 780, 768 $cm^{-1}$; $\delta$ ($CDCl_3$) 1·09, s, 1-Me; 1·34, s, 3-Me; 1·4–2·3, m, $CH_2$, s, CH; 4·08, d, *J* 10·5 Hz,

[4] Dean, F. M., Price, A. W., Wade, A. P., and Wilkinson, G. S., *J. Chem. Soc. C*, 1967, 1893.
[5] Coxon, J. M., Hartshorn, M. P., Mitchell, J. W., and Richards, K. E., *Chem. Ind. (London)*, 1968, 652.

$CH_AOR$; 4·29, d, $J$ 10·5 Hz, $CH_BOR$; 7·03, bs, NH; 7·2–8·0, m, aromatics; mass spectrum: $M^{+\bullet}$ 339·1823 ($C_{21}H_{25}NO_3$ requires 339·1833); *m/e* 339 (2·3%), 170 (11), 169 (84), 141 (18), 140 (21), 139 (77), 114 (10), 95 (24), 71 (16), 43 (100). The NaOH washings, after acidification and re-extraction into ether, yielded *p*-cresol (0·37 g) identical with authentic material on i.r., n.m.r., g.l.c. and co-g.l.c. Identical treatment of the $Na_2CO_3$ washings yielded a further trace of *p*-cresol. The $NaHCO_3$ washings yielded a brown gum.

The extracted aqueous phase of the urine was then saturated with ammonium sulfate, acidified to pH 3 with concentrated HCl and re-extracted with ethyl acetate. The organic extract was again washed sequentially with 5% $NaHCO_3$, 10% $Na_2CO_3$ and 5% NaOH solutions. Acidification of the $NaHCO_3$ washings revealed an acidic metabolite fraction (3·3 g) part of which (1·2 g) was treated with methyl iodide (1 ml) and anhydrous potassium carbonate in acetone at room temperature for 90 h. The resulting mixture (1·1 g) was shown by g.l.c. to contain three components in the ratio 24/61/7 in order of elution. Separation on an alumina (60 g) column enabled identification of the first two: elution with light petroleum yielded a fraction containing methyl benzoate (90% pure by g.l.c.), identical with authentic material on i.r., n.m.r., g.l.c. and co-g.l.c.; elution with 5% diethyl ether in light petroleum yielded a fraction containing *methyl 1,3-dimethyl-2-oxabicyclo[2,2,2]octane-3-carboxylate* (94% pure by g.l.c.) (5). $\nu_{max}$ 1760, 1735, 1270, 1210, 1195, 1120, 1070 $cm^{-1}$; $\delta$($CDCl_3$) 1·16, s, 1-Me; 1·42, s, 3-Me; 3·75, s, $COOCH_3$; mass spectrum: *m/e* 198 ($M^{+\bullet}$, 0·4%), 183·1020 ($M^{+\bullet}-15$, 2·6) ($C_{10}H_{15}O_3$ requires 183·1022), 140 (9), 139 (84), 95 (29), 81 (5), 71 (14), 43 (100).

*Control Urine*

The urine of a possum fed only fruit was extracted as above. From the saturated, acidified urine (1 l.) ethyl acetate extracted a product (0·54 g) shown by gas chromatography of its methyl ester to be benzoic acid.

*Synthesis of 1,3-Dimethyl-2-oxabicyclo[2,2,2]octane-3-methanol (2)*

Limonene (1·0 g) was oxidized with lead tetraacetate[4] to uroterpenol (27%) and the product (0·1 g) stirred with mercuric acetate (0·6 g) in anhydrous tetrahydrofuran (10 ml) at 55°. After 24 h 12% NaOH solution (5 ml) and a 0·5 M solution of sodium borohydride in 12% NaOH (5 ml) were added. This suspension was stirred for 1 h, saturated with sodium chloride and the organic layer removed to reveal 1,3-dimethyl-2-oxabicyclo[2,2,2]octane-3-methanol (2) (30%) shown by i.r. and co-g.l.c. to be identical to the neutral cineole metabolite from possum urine.

*Reduction of Methyl 1,3-Dimethyl-2-oxabicyclo[2,2,2]octane-3-carboxylate (5)*

The methylated metabolite from the 5% diethyl ether alumina column fraction (0·046 g) was refluxed in anhydrous tetrahydrofuran (20 ml) with lithium aluminium hydride (0·20 g) for 20 h to yield quantitatively 1,3-dimethyl-2-oxabicyclo[2,2,2]octane-3-methanol (2) shown by i.r. and co-g.l.c. to be identical with both the synthetic alcohol and the neutral metabolite.

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