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ESSENTIAL OIL METABOLISM IN THE KOALA
III NOVEL URINARY MONOTERPENOID LACTONES*[1]

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In a previous publication[2], the essential oil composition of the N.S.W. koala forage tree, Eucalyptus punctata ssp punctata was reported. After feeding on this leaf[1], the koala, Phascolarctos cinereus Goldfuss excreted urine from which three novel monoterpene lactones were isolated.

The first of these, for which the o-mentha-1,3-dien-1→8-olide (1) structure is proposed was obtained from the acid hydrolysis (1% H_2SO_4) of the acidic ($NaHCO_3$) fraction of the urine ether/methanol extract. Infrared absorption at 1750, 1665, 1570 and 1260 cm^{-1}, ultraviolet maxima at 226(ε 10400) and 297(ε 6000) nm and a molecular ion peak of 164.0832 indicated an αβ,γδ unsaturated $C_{10}H_{12}O_2$ lactone (α -71^0). Double irradiation and europium shift experiments enabled assignment of the p.m.r. signals as shown in Table 1. The mass spectrum revealed a molecular ion m/e 164(24%) losing CH_3COCH_3(58) and CH_3COHCH_3(59) to form fragments $C_6H_6CO^+$(106,100%) and $C_6H_5CO^+$(105,90%) respectively. Subsequent loss of CO formed $C_6H_6^+$(78,38%) and $C_6H_5^+$(77,20%) fragments respectively.

Hydrogenation of (1) with 10% Pd/C in EtOH for 10 mins yielded the dihydrolactone (2) showing ir absorption (C=O) at 1765 cm^{-1}, p.m.r. signals as shown in Table 1, and mass spectral fragmentation similar to (1) (m/e) 166(4%), 109(7%), 108(100%), 80(32%), 79(23%). Oxidation of (1) with DDQ in refluxing toluene for 20 hours yielded 3,3-dimethylphthalide (3) (m 71^0, ν_{max} 1760, 1610, 1280 cm^{-1}; λ_{max} 206(ε 7600), 228(ε 9190), 274(ε 1880) and 281(ε 1820) nm; m/e 162(8%), 148(9%), 147(100%), 104(4%), 91(17%), 77(5%), 76(5%); p.m.r. shifts as in Table 1.)

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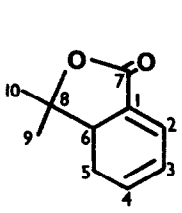
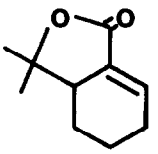
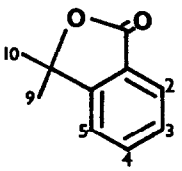
Treatment of o-acetylbenzoic acid[3] with MeMgBr using the Grignard lactonisation method of Berti, Marsili and Pacini[4] yielded synthetic (3) identical in all respects to the DDQ oxidation product of (1).



(1)



(2)



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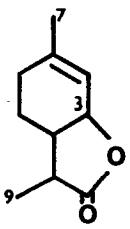
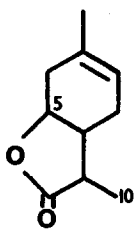


(4)



(5)

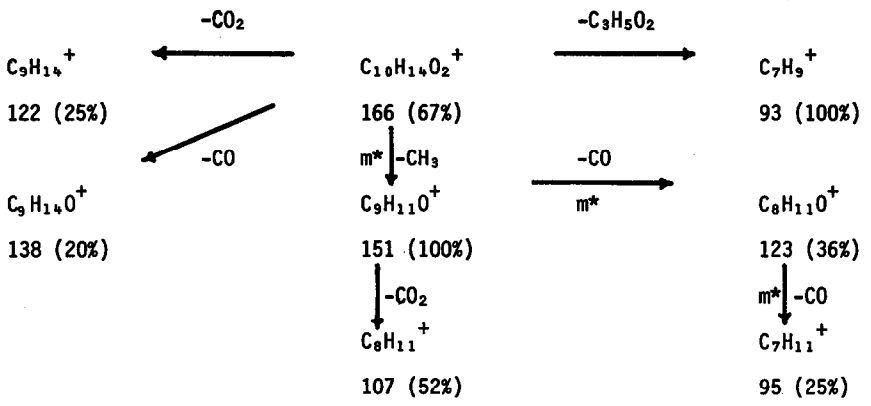
Table 1. The p.m.r. (δ) shifts for lactones (1) - (5) in $CDCl_3$.

	C 2	C 3	C 4	C 5	C 6	C 7	C 8	C 9	C 10
(1)	6.85	6.18	6.18	2.26	3.03	-	-	1.31	1.50
(2)	6.88	1.0-3.0	1.0-3.0	1.0-3.0	1.0-3.0	-	-	1.18	1.51
(3)*	7.84	7.47	7.65	7.39	-	-	-	1.63	1.63
(4)	5.55	4.93	1.0-3.0	1.0-3.0	1.0-3.0	1.74	2.38	1.29	-
(5)	5.70	1.0-3.0	1.0-3.0	4.70	1.0-3.0	1.79	2.90	-	1.20

*numbered here as a menthene derivative

The remaining lactones, for which the p-menth-1-en-8↔3-olide (4) and p-menth-1-en-8↔5-olide (5) structures are proposed were obtained from the neutral fraction of the ether-methanol extract. Although the isomers could not be isolated independently, glc-ms showed that each had molecular ions consistent with the $C_{10}H_{14}O_2^+$ ion and possessed identical fragmentations (Scheme 1). The loss of 73 is similar to the santonin series[5] loss of C_3H_5O thus suggesting the presence of an α-methyl, γ-lactone ring. The ir spectrum showed absorption at 1765, 1670, 1170, 970 and 945 cm^{-1}. From the p.m.r. spectra of mixtures of (4) and (5) in different proportions, assignments were made as in Table 1.

The structures of (1), (4) and (5) bear more resemblance to the ingested (+)-α- and (+)-β- pinene than to cineole and p-cymene, the other two major ingested components. Furthermore, the detoxication of cineole and p-cymene in other mammals proceeds via glucuronide ether[6]



Scheme 1

and glycine[6,7] conjugates respectively. Thus (1), (4) and (5) may be the elusive products of previous pinene ingestion studies[6,8]. The presence of substantial quantities of free or combined glucuronic acid in koala urine[9] was confirmed using Tollen's naphthoresorcinol[10] and Tomasić and Keglivić's[11] benzidine methods. The lower but daily increasing value for free glucuronic acid content obtained by the latter method, suggested that large amounts of labile glucuronide esters were being excreted. Thus it appears that lactones (1), (4) and (5) may arise from the cyclisation of carboxylic acids that are formed as hydrolysis products of glucuronide conjugates. In fact, many of the known detoxication product carboxylic acids[12] may be glucuronide ester hydrolysis products. For (1) to have been formed from α- or β-pinene, the unusual ring opening to the o-methane rather than the p-methane derivatives must be postulated. Further investigations on these detoxication processes are in progress.

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References

1 Part II. I.H. Eberhard, R.J. Pearse, J. McNamara and I.A. Southwell. Aust. J. Zool. 1975, in press.

2 I.A. Southwell, Phytochemistry, 12, 1341 (1973).

3 H.L. Yale, J. Amer. Chem. Soc., 69, 1547 (1947).

4 G. Berti, A. Marsili and P.L. Pacini, Ann. Chim. (Rome). 52, 1070 (1962).

5 D.G.B. Boocock and E.S. Waight, Chem. Comm. 90, (1966).

6 S.E. Wright, Univ. of Q'land Pub. Dept. Chem. 1 (25), 1 (1945).

7 J.M. Harvey, Univ. of Q'land Pub. Dept. Chem. 1 (23), 1 (1942).

8 J. Hämäläinen, Skand. Archiv. Physiol. 27, 141 (1912). (Chem. Abstr. 6 , 3412 (1912)).

9 N.T. Hinks and A. Bolliger, Aust. J. Sci. 19, 228 (1957).

10 S.W.F. Hanson, G.T. Mills and R.T. Williams, Biochem. J. 38, 274 (1944).

11 J. Tomasić and D. Keglivić, Anal. Biochem. 45, 164 (1972).

12 R.T. Williams, "Detoxication Mechanisms". Chapman and Hall, London (1959).