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ACETOPHENONES AND OTHER CONSTITUENTS FROM THE ROOTS OF *MELICOPE ERROMANGENSIS*

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Key Word Index—*Melicope erromangensis*; Rutaceae; roots; dimethylpyran acetophenones; quinolines; flavones; chemotaxonomy.

Abstract—Octandrenolone and five novel related dimethylpyran acetophenones have been isolated from the chloroform extract of roots of *Melicope erromangensis* and identified by spectroscopic studies and chemical correlations as *O*-methyloctandrenolone, (+)-*trans*-3‴,4‴-dihydro-3‴,4‴-dihydroxy-*O*-methyloctandrenolone, (+)-*trans*-3″,4″-dihydro-3″,4″-dihydroxy-*O*-methyloctandrenolone, (+)-*trans*-3‴,4‴-dihydro-3‴,4‴-dihydroxyoctandrenolone, and *trans*-3″,4″-dihydro-3″,4″-dihydroxyoctandrenolone. In addition, four known quinolines and three known flavones have been isolated.

INTRODUCTION

Melicope erromangensis T. Hartley ined. is a small tree up to 5 m high, probably endemic to Vanuatu (New-Hebrides). It is a new species which will be formally described by Hartley (unpublished results) in a revision of *Melicope*. In a continuation of our series on the chemical constituents of rutaceous plants from the Pacific Region, we report here on the isolation from the chloroform extract of roots of *M. erromangensis* and the structure determination of five novel acetophenones, related to octandrenolone (**1**) which has also been isolated from the plant material, together with four known quinolines and three known flavones.

RESULTS AND DISCUSSION

The chloroform extract of the roots of *M. erromangensis* yielded octandrenolone (**1**) [1], the five novel acetophenones (**2–6**) we describe here, four common alkaloids, dutadrupine (**8**) [2], skimmianine (**9**) [3], flindersiamine (**10**) [4] and kokusaginine (**11**) [5], and three known flavones, retusin (**12**), melisimplin (**13**) and 5-hydroxy-3,7-dimethoxy-3′,4′-methylenedioxyflavone (**14**). These last three compounds have already been isolated from *Melicope triphylla* [6].

All known compounds were identified by comparison of their spectral properties with those found in the literature. *O*-Methyloctandrenolone (**2**) is the major component of the chloroform extract (content: 0.07% of the dried plant material). The UV spectrum displays absorptions at λ_{max}^{MeOH} nm: 275 (sh.), 258, 246 (sh.), 203 (Table 1). The empirical formula was established by high-resolution spectrometry as $C_{19}H_{22}O_4$ (found: 314.1507; calcd; 314.1512). The ^{1}H NMR spectrum is very close to that of octandrenolone (**1**) (Table 2), revealing the presence of an acetophenone derivative bearing two nonsymmetrical fused dimethylpyran units [7]. The main differences lie in the disappearance of the hydroxyl signal at δ 14.00 and in the presence of a

Table 1. UV spectra of acetophenones **2–6**, **7a** and **7b** (λ_{max}^{MeOH}, nm)

2	**7a**	**3**	**5**	**7b**	**4**	**6**
275 (sh)	284 (sh)	285 (sh)	294 (sh)	284 (sh)	287 (sh)	295 (sh)
258	266	266	270	257	261	263
246 (sh)	224	224	227	235	235	233
203	207	204	204	203	204	205

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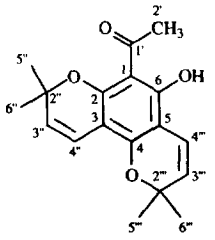

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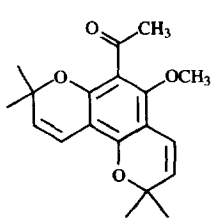

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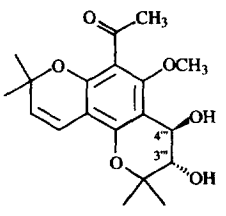

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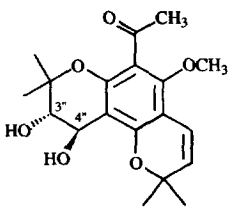

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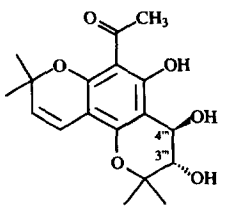

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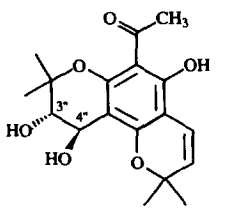

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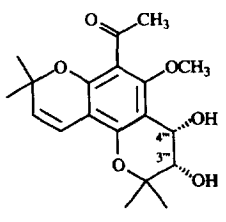
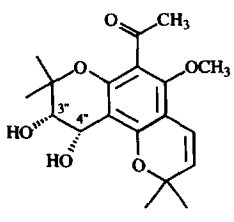


7a



7b

methoxyl signal at δ 3.76. These spectral data allow us to propose the structure of *O*-methyloctandrenolone for compound **2** in full agreement with the ^{13}C NMR spectrum (Table 3) for which all of the carbon resonances have been attributed unambiguously by COLOC [8, 9] and HETCORR [9, 10] correlation experiments. This structure has been confirmed by chemical correlation. Methylation of octandrenolone (**1**) (CH_3I–Me_2CO–KOH reflux, 2 hr) [11] leads to compound **2** in 95% yield.

(+)-*trans*-3‴,4‴-Dihydro-3‴,4‴-dihydroxy-O-methyloctandrenolone (**3**) was isolated as an amorphous yellow solid, $[\alpha]_D^{20}$ +20°($CHCl_3$; *c* 0.01) (content 0.005% from the dried material). The UV spectrum displays absorptions at λ_{max}^{MeOH} nm: 285 (sh.), 266, 204 (Table 1). The mass spectrum exhibits at (*m/z* 348.1565 (HR) $[M]^+$ $C_{19}H_{24}O_6$) i.e. an increase of 34 amu corresponding to the addition of H_2O_2 when compared with *O*-methyloctandrenolone (**2**). In contrast to the preceding compounds, the ^1H NMR spectrum of **3** exhibits only one AB system (J = 10.0 Hz) typical of a chromene unit, whereas a second AB system (J = 7.0 Hz) at δ 3.73 and 4.72 suggests the presence of a dihydrodihydroxydimethylpyran unit. Cross-peaks observed in the COLOC spectrum between the signals of the methoxyl-bearing carbon (C-6) and the benzylic proton of the dihydrodihydroxydimethylpyran unit (H-4‴) provides evidence for the location of the diol next to the methoxyl group. Compound **3** remains unchanged upon treatment with acetone (Me_2CO–HCl, 20°, 18 hr) under conditions known to produce acetonides from *cis*-chromene derived diols [12]. This suggests a relative *trans* configuration of the natural product, in good agreement with the lack of a nOe between H-3‴ and H-4‴. In order to determine unambiguously the relative stereochemistry of compound **3**, *cis*-selective oxidation (OsO_4–pyridine, 20°, 3 hr) [13, 14] of **2** was performed. This reaction led to two oxidation products, **7a** and **7b**, in 21% and 18% yield, respectively. Both are different from **3** and exhibit a 5.0 Hz coupling constant (Table 2) between the CH signals of the dihydrodihydroxy-dimethylpyran unit. The structure of compound **3** is therefore established as (+) - *trans* - 3‴,4‴ - dihydro - 3‴,4‴ - dihydroxy - *O* - methyloctandrenolone. The UV spectra of compounds **7a** and **7b** are different (Table 1), but that of **7a** is identical to that of **3**, demonstrating a similar location of the diol next to the methoxyl on both compounds.

(+)-*trans*-3″,4″-Dihydro-3″,4″-dihydroxy-*O*-methyloctandrenolone (**4**) was isolated as an amorphous yellow solid, $[\alpha]_D^{20}$ +8.3° ($CHCl_3$; *c* 0.012) (contents: 0.003% of the dried material). Its mass spectrum is almost identical with that of compound **3** and exhibits a $[M]^+$ *m/z* 348.1562 (HR) corresponding to the empirical formula $C_{19}H_{24}O_6$. Its ^1H and ^{13}C NMR data (Tables 2 and 3) are closely related to those of compound **3** and differs only in slight chemical shift variations. The coupling constant (J = 7.0 Hz) of the CH signals of the dihydrodihydroxydimethylpyran unit gives evidence for the *trans* configuration of the diol. Furthermore, the UV spectrum of compound **4** is superimposable on that of **7b** (Table 1) indicating the location of the hydroxyl groups at positions 3″ and 4″. The structure of compound **4** is therefore depicted as (+)-*trans*-3″,4″-dihydro-3″,4″-dihydroxy-*O*-methyloctandrenolone.

Compounds **5**, $[\alpha]_D^{20}$ +6.6° ($CHCl_3$; *c* 0.006), and **6**, $[\alpha]_D^{20}$ = 0° ($CHCl_3$; *c* 0.004), were isolated in very small amounts (contents 0.0007% and 0.0006% from the dried plant material, respectively). On mass spectrometry both exhibit $[M]^+$ at *m/z* 334 corresponding to the empirical formulae $C_{18}H_{22}O_6$. Both, therefore, appear to be dihydrodihydroxyoctandrenolones. Their ^1H NMR spectra (Table 2) exhibit a 7.0 Hz coupling constant between the CH signals of the dihydrodihydroxy-dimethylpyran unit and is once more indicative of a *trans* configuration. The UV spectra of compounds **5** and **6** are identical to those of **7a** and **7b** respectively (Table 1). The structure of (+)-*trans*-

Table 2. ^{1}H NMR spectra of acetophenones **2–6**, **7a** and **7b** (300 MHz, $CDCl_3$/TMS, δ ppm, J in Hz)

H	2	3	4	5	6	7a	7b
Co-Me-2′	2.51 *s*	2.53 *s*	2.50 *s*	2.62 *s*	2.68 *s*	2.53 *s*	2.50 *s*
OH-6	–	–	–	14.0 *s*	14.0 *s*	–	–
OMe-6	3.76 *s*	3.84 *s*	3.75 *s*	–	–	3.83 *s*	3.75 *s*
H-3″	5.52*	5.59	3.75	5.50	3.82	5.51	3.77
	d: 10.0	*d*: 10.0	*m*	*d*: 10.0	*d*: 7.0	*d*: 10.0	*t*: 5.0
H-4″	6.49†	6.56	4.75	6.67	5.02	6.60	4.97
	d: 10.0	*d*: 10.0	*dd*: 7.0,2.0	*d*: 10.0	*d*: 7.0	*d*: 10.0	*dd*: 5.0,2.0
OH-3″	–	–	1.70	–	3.24	–	3.12
			br s§		*br s*§		*d*: 5.0§
OH-4″	–	–	2.72	–	4.27	–	3.70
			br s§		*br s*§		*d*: 2.0§
H-3‴	5.54*	3.73	5.55	3.78	5.43	3.76	5.55
	d: 10.0	*d*: 7.0	*d*: 10.0	*d*: 7.0	*d*: 10.0	*t*: 5.0	*d*: 10.0
H-4‴	6.60†	4.72	6.50	4.73	6.58	4.95	6.50
	d: 10.0	*d*: 7.0	*d*: 10.0	*d*: 7.0	*d*: 10.0	*dd*: 5.0,2.0	*d*: 10.0
OH-3‴	–	–	–	2.55	–	3.13	–
				br s§		*d*: 5.0§	
OH-4‴	–	–	–	3.55	–	3.94	–
				br s§		*d*: 2.0§	
Me-5″		1.41	1.26	1.48	1.31	1.42	1.44
		s	*s*	*s*	*s*	*s*	*s*
	1.43‡						
Me-6″	*s*(6H)	1.45	1.50	1.52	1.48	1.43	1.46
		s	*s*	*s*	*s*	*s*	*s*
Me-5‴		1.25		1.34		1.31	1.32
		s		*s*		*s*	*s*
	1.57‡		1.45		1.50		
Me-6‴	*s*(6H)	1.48	*s*(6H)	1.56	*s*(6H)	1.47	1.52
		s		*s*		*s*	*s*

*,†,‡ Assignments may be reversed in the same column.
§ D_2O exchangeable.

Table 3. ^{13}C NMR spectra of acetophenones **2–6** (75 MHz, $CDCl_3$/TMS, δ ppm)

C	2	3	4	5	6
1	117.5	117.3	118.3	116.7*	116.7
2	151.2*	151.6	150.3*	157.8**	154.7
3	106.4†	106.4	108.1	105.7	105.4
4	150.3*	149.7	153.4*	155.4**	156.3
5	108.0†	109.7	108.1	105.7	105.4
6	153.5	156.5	153.8	161.3	164.6
OCH_3	63.4	62.5	63.2	–	–
1′	200.7	200.8	200.6	203.6	203.8
2′	32.5	32.5	32.4	33.4	33.0
2″	76.7‡	77.0	79.0	79.2	79.1
3″	127.6	127.6	74.9	124.9	70.4
4″	116.1§	116.2	67.7	116.1*	62.4
5″	27.8	27.3	19.5	28.5	23.2
6″	27.8	28.0	25.7	29.7	24.1
2‴	77.0‡	78.6	77.9	79.8	78.2
3‴	127.6	75.0	127.1	74.6	124.7
4‴	116.6‡	67.7	116.5	67.4	116.7
5‴	27.8	19.3	28.0	20.0	27.8
6‴	27.8	25.8	28.0	25.7	29.7

*,†,‡,§ Assignments may be reversed on the same column.

3‴,4‴-dihydro-3‴,4‴-dihydroxyoctandrenolone is therefore assigned to compound **5** and that of *trans*-3″,4″-dihydro-3″,4″-dihydroxyoctandrenolone to compound **6**.

It should be noted that, unfortunately, the absolute configurations of compounds **3**, **4**, **5** and **6** could not be determined because of the small quantity of material isolated and the lack of relevant CD models in these series.

From a chemotaxonomic point of view, it should be noted that acetophenones have been so far only isolated from *Rutaceae* species belonging to the sub-family Rutoideae (tribes Zanthoxyleae and Boronineae) except in the case of *Acronychia* which was placed in the subfamily Toddalioideae [1]. However, the division between these two subfamilies is now largely discredited [15]. The chemical constitution of *M. erromangensis* (acetophenones, flavones, alkaloids) is very close to those of the other *Melicope* species [1].

EXPERIMENTAL

Roots extractions were carried out using a Soxhlet apparatus. Spectra were recorded on the following instruments: UV, Shimadzu UV 160A; MS, Nermag R-10-10H in electron impact (70 eV); ^{1}H NMR, Bruker AC 300 (300 MHz); ^{13}C NMR, Bruker AC 300 (75 MHz); ^{1}H–^{13}C HETCORR, COLOC and NOESY experiments were performed on a Bruker AC 300 using the standard Bruker microprograms.

Plant material. The plant material used in this study was collected on a low altitude slope of the primary forest of Erromango island (New-Hebrides). An Herbarium sample (Sam Channel 198) is kept in the herbarium of the Center ORSTOM in Noumea (New-Caledonia).

Extraction. Dried powdered roots (300 g) were defatted by extraction with petrol (40–60°) and extracted with $CHCl_3$ and MeOH.

Separation. The $CHCl_3$ extract (1.5 g) was submitted to CC using silica gel 60 Merck (particle size: 0.063–0.200 mm) packed in *n*-hexane. Elution was performed with *n*-hexane containing increasing amounts of $CHCl_3$, then $CHCl_3$ containing increasing amounts of EtOAc. Frs were monitored by TLC and those containing comparable mixts were continued and purified by centrifugal prep. TLC (silica gel 60 PF_{254}, solvent petrol–EtOAc gradients) and by prep. TLC (silica gel 60 F_{254} with concentrating zone).

The acetophenones **2**–**6** were isolated as yellow amorphous solids.

O-Methyloctandrenolone (**2**). $C_{19}H_{22}O_4$ (found: 314.1507; calcd: 314.1512). UV: Table 1; EI-MS *m/z*: 314 $[M]^+$; ^{1}H NMR: Table 2; ^{13}C NMR: Table 3.

Methylation of **1**. A soln of **1** (35 mg), KOH (20 mg) and MeI (0.07 ml) in 3 ml Me_2CO was heated under reflux for 2 hr, hydrolysed with H_2O (10 ml) then extracted with $CHCl_3$. The organic layer was dried over dry Na_2SO_4, filtered and evapd *in vacuo* to give **2** (35 mg) in 95% yield.

(+)-*trans*-3‴,4‴-*Dihydro*-3‴,4‴-*dihydroxy*-*O*-*methyloctandrenolone* (**3**). $C_{19}H_{24}O_6$ (found: 348.1565; calcd: 348.1572). $[\alpha]_D^{20} = +20°$ ($CHCl_3$; *c* 0.01). EI-MS *m/z*: 348 $[M]^+$; ^{1}H NMR: Table 2; ^{13}C NMR: Table 3.

cis-Oxidation of **1**. Os_2O_4 (40 mg) was added to a soln of **2** (46 mg) in pyridine (1 ml) and stirred for 3 hr at 20°. A soln of satd aq. $Na_2S_2O_5$ (10 ml) was added then the mixt. was extracted with $CHCl_3$. The organic layer was dried over dry Na_2SO_4, filtered and evapd *in vacuo*. Prep. TLC (silica gel 60 F254, solvent *n*-hexane-EtOAc 1:1) of the residue led to the isolation of **7a** and **7b** in 12% and 15% yield, respectively. ^{1}H NMR and ^{13}C NMR data of **7a** and **7b** are given in Tables 2 and 3.

(+)-*trans*-3″,4″-*Dihydro*-3″,4″-*dihydroxy*-*O*-*methyloctandrenolone* (**4**). $C_{19}H_{24}O_6$ (Found: 348.1562; Calcd; 348.1572). $[\alpha]_D^{20} = +8.3°$($CHCl_3$; *c* 0.012). EI-MS *m/z*: 348 $[M]^+$; UV: Table 1; ^{1}H NMR: Table 2; ^{13}C NMR: Table 3.

(+)-*trans*-3‴,4‴-*Dihydro*-3‴,4‴-*dihydroxyoctandrenolone* (**5**). $C_{18}H_{22}O_6$ (found: 334.1436; calcd: 334.1416). $[\alpha]_D^{20} = +6.6°$ ($CHCl_3$; *c* 0.006). EI-MS *m/z*: 334 $[M]^+$; UV: Table 1; ^{1}H NMR: Table 2; ^{13}C NMR: Table 3.

trans-3″,4″-*Dihydro*-3″,4″-*dihydroxyoctandrenolone* (**6**). $C_{18}H_{22}O_6$ (found: 334.1451; Calcd: 334.1416). $[\alpha]_D^{20}$ 0.0° ($CHCl_3$; *c* 0.004). EI-MS *m/z*: 334 $[M]^+$; UV: Table 1; ^{1}H NMR: Table 2; ^{13}C NMR: Table 3.

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REFERENCES

1. Quader A., Armstrong, J. A., Gray A. I., Hartley T. G. and Waterman P. G. (1991) *Biochem. Syst. Ecol.* **19**, 171.
2. Baudouin G., Tillequin F., Koch M., Pusset J. and Sevenet T. (1981) *J. Nat. Prod.* **44**, 546.
3. Sangster A. W. and Stuart K. L. (1965) *Chem. Rev.* **65**, 69.
4. Vaquette J., Hifnawy M. S., Pousset, J. L., Fournet, A., Bouquet A. and Cavé A. (1976) *Phytochemistry* **15**, 743.
5. Khalid S. A. and Waterman P. G. (1981) *Phytochemistry* **20**, 2761.
6. Higa M., Oshiro T., Ogihara K. and Yogi S. (1990) *Yakugaku Zasshi* **110**, 822.
7. Timar, T., Hosztafi S. and Jaszberenyl J. C. (1988) *Acta. Chem. Hung.* **125**, 617.
8. Kessler H., Griesinger G., Zarbock J. and Loosli H. R. (1984) *J. Magn. Reson.* **57**, 331.
9. Derome A. E. (1983) *Nat. Prod. Rep.* **95**, 111.

10. Benn R. and Gunter H. (1983) *Angew. Chem.* **95**, 381.
11. Baudouin G., Tillequin F., Koch M., Vuilhorgne M., Lallemand J. H. and Jacquemin H. (1983) *J. Nat. Proc.* **46**, 681.
12. Glotter, E. and Zviely M. (1984) *J. Chem. Soc. Perkin Trans. I*, 1345.
13. Brown R. F. C., Hobbs J. J., Hugues G. K. and Ritchie E. (1954) *Aust. J. Chem.* **7**, 348.
14. Jennings R. C. and Ottridge A. P. (1984) *J. Chem. Soc. Perkin Trans. I*, 2345.
15. Ng K. M., But P. P., Gray A. T., Hartley T. G., Kong Y. C. and Waterman P. G. (1987) *Biochem. Syst. Ecol.* **15**, 587.