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SESQUITERPENOID CONSTITUENTS OF *ENTANDROPHRAGMA CYLINDRICUM*

WŁODZIMIERZ M. DANIEWSKI, WALDEMAR ANCZEWSKI, MARIA GUMUŁKA, WITOLD DANIKIEWICZ, ULLA JACOBSSON* and TORBJORN NORIN*

Institute of Organic Chemistry, Polish Academy of Sciences, Kasprzaka 44, 01-224 Warsaw, Poland; *Royal Institute of Technology, Department of Organic Chemistry, S-100 44 Stockholm, Sweden

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Key Word Index—*Entandrophragma cylindricum*; Meliaceae; sesquiterpenes; 10β-hydroxy-calamenene; 10β-hydroxy-copa-4-ene; 2α-hydroxy-copa-3-en; T-cadinol; ledol; mustakon.

Abstract—A petrol extract of *Entandrophragma cylindricum* afforded six sesquiterpenes. Two of them, 3-hydroxy-copa-2-en and 2α-hydroxy-copa-3-en, are new. 10-Hydroxy-*trans*-calamenene, T-cadinol, ledol and mustacon have already been reported. The structures of all these compounds were substantiated by extensive NMR experiments. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

In searching for antifeedants against storage pests, we examined a petrol extract obtained from the bark of *Entandrophragma cylindricum* C. DC. The extract showed good antifeedant activity against *Trogoderma granarium*, *Tribolium confusum* and *Sitophilus granarius* [1], so it was of interest to use to isolate the compounds responsible for the activity. The tree belongs to the Meliaceae, which comprises *Azadirachta indica* from which the very potent antifeedant azadirachtin was isolated. *E. cylindricum* has already been investigated and the isolation and structural elucidation of protolimonoids sapelins B [2], C, [3] D [3], E. [3] and F [4] an a derivative of canodollein, (99) 3-(2′-hydroxy), have been reported [5, 6]. In the present study, gradient elution chromatography, followed by preparative HPLC, led to the isolation of six sesquiterpenes (**1**–**6**, numbered in order of increasing polarity) of which two are new.

RESULTS AND DISCUSSION

Compound **1** gave a molecular ion in its mass spectrum at *m/z* 220. The HR mass spectrum of **1** pointed to the formula $C_{15}H_{24}O$. The IR spectrum of **1** exhibited a hydroxyl absorption band at 3383 cm^{-1} and a C=C double bond band at 1658 cm^{-1}. These facts were confirmed by the ^{13}C NMR spectrum of **1**, which contained signals at 85.99 (*s*), 126.36 (*d*) and 140.17 (*d*). The above data led to the assumption that **1** was a tricyclic sesquiterpene. The ^{1}H and ^{13}C NMR spectra revealed that **1** possessed four methyl groups in its molecule. The lowest field signal was assigned to a methyl group attached to a carbon atom bearing a hydroxyl group. The second signal at δ 0.93 was assigned to a methyl group attached to a quaternary carbon atom. The remaining methyl groups formed a six-proton doublet ($J = 6.4$ Hz) which was assigned to an isopropyl group. The ^{1}H NMR spectrum of **1** measured in benzene-d_6 revealed signals which are characteristic of ylangene or copaene skeletons i.e. δ 2.47 (*brs*, H-1), 1.84 (*dd*, H-2) and 2.03 (*dd*, H-6) (Table 1). A similar compound, copan-4-ol (**2**) was isolated from *Ocimum americanum* [7] and in its ^{13}C and ^{1}H NMR spectra the shift of C-4 was δ 69.2 and H-15 δ 1.42. The presence of a C-2 and C-3 double bond caused a further downfield shift of the C-4 signal to δ 85.99 and the C-15 methyl signal to δ 1.43. The ^{1}H and ^{13}C NMR data are presented in Table 1. The assignments were substantiated by 2D ^{1}H–^{1}H and ^{1}H–^{13}C COSY correlations. The chemical shifts of carbons C-5 and C-1 allowed us, after reference to the literature [8], to assign the copaene structure to **1**.

Extensive spectroscopic investigations of **3** and **4** allowed us to identify them as mustakone [9] and T-cadinol [10], respectively.

Similarly, spectroscopic investigations of the next sesquiterpene (**5**) allowed us to identify it as (+)-ledol, which was isolated earlier from *Ledum palustre* and other species [11–13]. Later, the isolation of (−)-ledol from *Piper clussi* was reported [14]. Compound **5** showed a specific rotation of −5.5° when it was measured in $CHCl_3$ solution in agreement with the last data, so we thought that this was another example of (−)-ledol. Compound **5** showed very strong antifeedant activity against storage pests (unpublished work) and it was, therefore, of great interest to check the antifeedant activity of (+)-ledol, the enantiomer isolated from *L. palustre*, a plant which is still used in villages in Poland

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data for compounds **1** and **6** (TMS as int. standard)

	^{1}H NMR			^{13}C NMR	
C	**1** ($CDCl_3$)	**1** (C_6D_6)	**6** ($CDCl_3$)	**1** ($CDCl_3$)	**6** ($CDCl_3$)
1	2.28 *dd*	2.03 *dd*	2.21 *ddd*	39.2 *d*	43.8 *d*
2	6.41 *dd*	6.20 *dd*	4.32 *brs*	140.2 *d*	70.5 *d*
3	5.60 *ddd*	5.60 *ddd*	5.37 *m*	126.4 *d*	118.5 *d*
4	—	—	—	86.0 *s*	148.4 *s*
5	1.85 *dd*	1.84 *dd*	1.60 *m*	56.2 *d*	55.3 *d*
6	2.28 *br s*	2.47 *br s*	1.86 *br s*	45.4 *d*	41.6 *d*
7	1.60 *m*	1.60 *m*		45.1 *d*	44.4 *d*
8	1.60 *m*	1.60 *m*	1.6 *m* (5H)	21.6 *t*	21.7 *t*
9	1.75 *m*	1.60 *m*		37.7 *t*	36.4 *t*
10	—	—	—	48.8 *s*	48.6 *s*
11	1.60 *m*	1.60 *m*	1.55 *m*	32.1 *d*	32.1 *d*
12	0.88 *d*	0.85 *d*	0.894*d*	20.0 *q*[a]	19.5 *q*[b]
13	(6H)	(6H)	(6H)	19.3 *q*[a]	20.0 *q*[b]
14	0.93 *s*	0.86 *s*	0.85 *s*	22.3 *q*	18.9 *q*
15	1.43 *s*	1.40 *s*	1.74 *dd*	21.6 *q*	22.7 *q*

J(HZ) **1**: 1α, 2α = 2.1; 2α, 6α = 6.4; 4, 5 = 8.7; 4, 6α = 2.1; 5, 6α = 6.4; 12, 11 = 6.4; 13, 11 = 6.4; **6**: 4, 15 = 1.5; 2α, 6α = 6.2; 12, 11 = 6.7; 13, 11 = 6.7; 5β, 6α = 3.52; 4, 6α = 3.5.

[a,b]Signals within column can be interchanged.

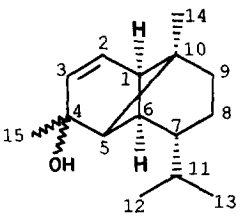
to scare off moths from wardrobes. *L. palustre* was collected in the Mazurian Lake District in Poland and the ledol was isolated. To our surprise, its specific rotation measured in $CHCl_3$ was exactly the same as that isolated from *E. cylindricum*, i.e. −5.7°. Therefore, we checked the literature again to find out that originally the specific rotation of ledum camphor (ledol) was measured in ethanol, and it was equal to +4°. Our measurement performed in ethanol (+1.1°) confirmed that, and thus it is clear that only one enantiomer of ledol has been isolated from natural sources and that its specific rotation depends very much on the solvent used for the measurement.

Compound **6** on mass spectrometry gave rise to a molecular ion at *m/z* 220. Its HR mass spectrum pointed to the formula $C_{15}H_{24}O$. The IR spectrum of **6** exhibited a hydroxyl band at 3345 cm^{-1} and a carbon–carbon double bond band at 1660 cm^{-1}. These findings were confirmed by the ^{13}C NMR spectrum of **6**, which showed the signal of a carbon of δ 70.5 (*d*) and two signals at δ 118.46 (*d*) and 148.45 (*s*). On considering the data above, it was assumed that **6** was a sesquiterpene alcohol containing a secondary hydroxyl group and a double bond. The ^{1}H NMR spectrum supported this assumption as signals of four methyl groups were visible. The lowest field methyl signal was broadened by an allylic coupling with a vinyl proton; therefore, it was attached to a quaternary sp^2 carbon. Two methyl signals were doublets with J = 6.71 Hz and were assigned to an isopropyl group. The last methyl signal was a singlet; therefore, it was attached to a quaternary carbon atom. The ^{1}H NMR spectrum of **6** exhibited a broad singlet at δ 1.86, which, in conjunction with the presence of a signal at δ 2.21 (*ddd*, H-6), allowed us to assign the skeleton of either copaen or ylangen to **6**. All of the proton and carbon assignments presented in Table 1 were substantiated by 2D ^{1}H–^{1}H and ^{1}H–^{13}C COSY NMR spectra. Some single decouplings were also performed. Even with full NOESY spectra, it was not possible to distinguish between ylangen (β-isopropyl group) or copaen (α-isopropyl group); however, the chemical shifts of C-5 and C-1 univocally allowed us to choose structure **6** (copaen) after consultation of the literature [8].

Spectroscopic investigations of **7** led to the elucidation of its structure as 10-hydroxycalamenene. A compound possessing the same ^{1}H NMR spectrum was isolated from *Siparuna macrotepala* [15], but the authors neither defined its stereochemistry at C-10 nor reported its specific rotation. The signals of both methyls of the isopropyl group in the spectrum of **7** are shifted downfield by 0.06 ppm, in comparison with the same signals of *trans*-calamenene, which is caused by the presence of an oxygen atom in the molecule. Attempts to prepare a crystalline derivative of **7** failed, and the unavailability of both *cis* and *trans* isomers did not allow us to make any firm assumptions regarding the stereochemistry of asymmetric centres at C-7 and C-10. However, since **7** is an aromatized derivative of T-cadinol (**4**) (which we isolated from the same plant), we can assume that the asymmetric centres at C-7 and C-10 possess the same stereochemistry, i.e. the hydroxyl group is at the same side of the molecule as the isopropyl methyls, which caused their downfield shift.

EXPERIMENTAL

The bark of *E. utile* was collected in the primeval forest in Zaire near Kisangani in 1990. The tree was identified by Prof. F. Szafrański, University of Kisangani, according to ref. [16]. The dry bark (90 g) after exhaustive extraction with petrol gave an extract (15.9 g) which was subjected to CC on silica-gel (hexane–EtOAc gradient). The chromatography was



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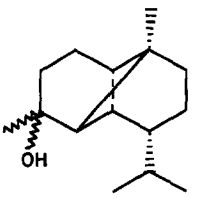

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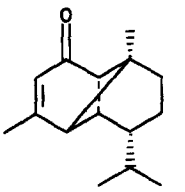

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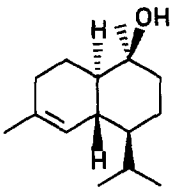

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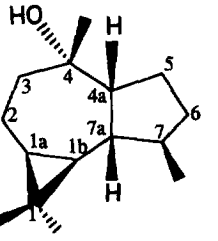

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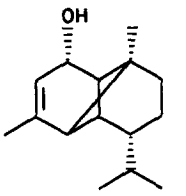

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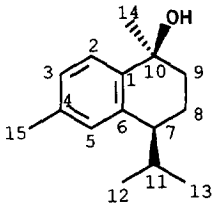

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monitored by TLC (hexane–$CHCl_3$–EtOAc, 8:1:1). The fr. possessing $R_f = 0.3$ (2.84 g) was subjected to semi-prep. HPLC using a set of 5 columns (8 × 300 mm) filled with LiChrosorb Si60 (10 μm) joined in series. The column was eluted with hexane–$CHCl_3$–EtOAc (12:1:1) at 6 ml min^{-1}. The chromatography was monitored with a RI detector. The following peaks were collected and evapd to give:

Copa-2-en-4-ol (**1**, 114 *mg*, $K' = 1.56$). Oil; $[\alpha]_D^{20}$ + 39.55° ($CHCl_3$; *c* 0.3); IR ν_{max}^{film} cm^{-1}: 3382, 1658, 1465, 1371; ^1H and ^{13}C NMR: Table 1; HR-MS: $[M]^+$ 220.18400 calc. for $C_{15}H_{24}O$ 220.18271; MS 70 eV *m/z* (rel. int.): 220 (5), 202 (8), 191 (3), 177 (33), 159 (67), 150 (18), 145 (30), 159 (67), 150 (18), 145 (30), 131 (52), 119 (100), 105 (67), 93 (55), 81 (42), 69 (23), 55 (23), 43 (54).

Mustakon (**3**, 103 *mg*, $K' = 2.60$). Oil; $[\alpha]_D^{20}$ − 51.6° ($CHCl_3$; *c* 0.2); ^1H and ^{13}C NMR the same as those published [9]; HR-MS $[M]^+$ 218.16549, calc. for $C_{15}H_{22}O$ 218.16706.

T-cadinol (**4**, 95 *mg*, $K' = 2.91$). Mp 44–46°; $[\alpha]_D^{20}$ + 2.1° ($CHCl_3$; *c* 0.5); ^1H and ^{13}C NMR: the same as those published [10]; HR-MS $]M - H_2O]^+$ 204.18699, calc. for $C_{15}H_{24}$ 204.1878.

Ledol (**5**, 220 *mg*, $K' = 3.43$). Mp 104°; $[\alpha]_D^{20}$ − 5.5° ($CHCl_3$; *c* 1.0); $[\alpha]_D^{20}$ + 1.1° (EtOH; *c* 1.0); ^1H and ^{13}C NMR: the same as those published [14]; HR-MS $[M]^+$ 222.19902, calc. for $C_{15}H_{26}O$ 222.19836.

Copa-3-en-2α-ol (**6**, 42 *mg*, $K' = 3.69$). Mp 57°; $[\alpha]_D^{20}$ − 47.6° ($CHCl_3$; *c* 0.5); IR ν_{max}^{film} cm^{-1}: 3345, 1669, 1634, 1442, 1373; ^1H and ^{13}C NMR: Table 1; HR-MS $[M]^+$ 220.18129, calc. for $C_{15}H_{24}O$ 220.18271; MS 70 eV *m/z* (rel. int.): 220 (20), 205 (23), 202 (17), 191 (97).

Calamenene-10β-ol (**7**, 22 *mg*, $K' = 4.65$). Oil; $[\alpha]_D^{20}$ − 33.7° ($CHCl_3$; *c* 0.5); HR-MS $[M]^+$ 218.16714, calc. for $C_{15}H_{22}O$ 218.16706.

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REFERENCES

1. Błoszyk, E., Szafrański, F., Drożdż, B. and Kamal, A.-S. (1995) *J. Herbs, Spices Med. Plants* **3**, 25.
2. Chan, W. R., Taylor, D. R. and Yee, T. (1970) *J. Chem. Soc.* (*C*) 311.
3. Chan, W. R., Taylor, D. R. and Yee, T. (1971) *J. Chem. Soc.* (*C*) 2662.

4. Halsall, T. G., Wragg, K., Connolly, J. D., MacLellan, M. A., Bredell, L. D. and Taylor, D. A. H. (1977) *J. Chem. Res. (S)* 154, (M) 1727.
5. Okorie, D. A. and Taylor, D. A. H. (1977) *Phytochemistry* **16**, 2029.
6. Taylor, D. A. H. (1984) *Fortschr. Chem. Org. Naturst.* **45**, 1.
7. Upadhyay, R. K., Misra, L. N. and Singh, G. (1991) *Phytochemistry* **30**, 691.
8. De Buyck, L. F., De Pooter, H. L., Schamp, N. M., De Bruyn, A., Shang, W., Budesinsky, M. and Motl, O. (1989) *Flavour Fragr. J.* **4**, 53.
9. Nyasse, B., Ghogomu Tih, R., Sondengham, B. L., Martin, M. T. and Bodo, B. (1988) *Phytochemistry* **27**, 3319.
10. Cleason, P. and Samuelsson, G. (1991) *Planta Med.* **57**, 352.
11. Pakrashi, S. C., Dastiar Gosh, P. P., Chakrabarty, S. and Achari, B. (1980) *J. Org. Chem.* **45**, 4765.
12. Dolejs, L., Motl, O., Soucek, M., Herout, V. and Sorm, F. (1960) *Collect. Czech. Chem. Commun.* **25**, 1483.
13. Dolejs, S. J. and Sorm, F. (1959) *Tetrahedron Letters* **17**, 1.
14. Koul, S. K., Taneja, S. C., Malhotra, S. and Dhar, K. L. (1993) *Phytochemistry* **32**, 478.
15. El-Seedi, H., Ghia, F. and Torssell, K. B. G. (1994) *Phytochemistry* **35**, 1495.
16. Staner, P. and Gilbert, G. (1958) *Flore du Congo-belge et du Ruanda–Burundi* **VII**, 182.