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(-)-EUDESMA-1,4(15),11-TRIENE FROM THE ESSENTIAL OIL OF *CALLITRIS INTRATROPICA*

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Key Word Index—*Callitris intratropica*; Coniferae; essential oil; sesquiterpene; (-)-eudesma-1,4(15),11-triene.

Abstract—A new eudesmane sesquiterpene was isolated from the essential oil of *Callitris intratropica*. The structure was established as (-)-eudesma-1,4(15),11-triene by means of spectroscopic methods and enantioselective gas chromatography.

INTRODUCTION

Callitris intratropica commonly called *blue cypress* is of the cypress pine family (Cupressaceae) belonging to the Coniferae genus [1]. Although a native of West Australia, it is widely grown in Europe and the tree is noted for its large percentage of fragrant oil [1, 2]. Isolation of guaiol (**1**), α-, β- and γ-eudesmol (**2–4**), and cryptomeridiol (**5**) from the oil has been reported [3]. We report now the isolation and the structural elucidation of (-)-eudesma-1,4(15),11-triene (**6**) from the oil.

RESULTS AND DISCUSSION

The investigation of the essential oil of *C. intratropica* by gas chromatography-mass spectrometry (GC–MS) led to the identification of a series of known sesquiterpenes including β-elemene (**7**), selina-4,11-diene (**8**), α-guaiene (**9**), α-guaioxide (**10**), β-selinene (**11**), α-selinene (**12**), α-bulnesene (**13**), α-guaiol (**1**), β-eudesmol (**3**), γ-eudesmol (**4**), and bulnesol (**14**), by comparison of the mass spectra and GC retention indices with those of reference compounds. Furthermore, a sesquiterpene lactone, dihydrocolumellarin (guaianolide **15**), which was described before as a constituent of *Callitris columellaris* (incomplete NMR data) [4], was identified and its *trans*-configuration confirmed by the NOESY technique. An unknown sesquiterpene hydrocarbon with a molecular mass of 202 ($C_{15}H_{22}$) eluted as a major peak of the hydrocarbon fraction in the GC just after **7** from an unpolar stationary phase (dimethylpolysiloxane). The essential oil was submitted to silica column chromatography at low temperature yielding several hydrocarbon fractions and some oxygenated sesquiterpenes. Further treatment of one of the hydrocarbon fractions using preparative GC [4] allowed the isolation of compound **6** as a pure material. Together with **6** four other components were isolated and identified as β-elemene (**7**), α-guaiene (**9**), α-guaioxide (**10**) [5] and guaianolide (**15**) [4].

Compound **6** was investigated using 1- and 2D NMR techniques. The ^{13}C NMR spectrum together with the DEPT technique permitted the identification of fifteen carbon atoms: six sp^2 carbons, two methyl groups, six methylene, four methine and three quaternary carbons. Among the olefinic carbons, those at δ 148.05 (*s*) and 108.70 (*t*) were typical of isopropenyl groups (C-11 and C-12) while those at δ 150.99 (*s*) 106.40 (*t*) suggested an exocyclic double bond. The ^1H-NMR spectrum confirmed the presence of the isopropenyl group: two broad singlets at δ 4.72 and 4.74 showing coupling correlations (^1H–^1H COSY diagram) with the methyl group at 1.76. The exocyclic double bond was also confirmed by the proton signals at δ 4.59 (1H, *br d*, $J = 1.6$ Hz) and 4.84 (1H, *br d*, $J = 1.6$ Hz). The compound was then assumed to have a bicyclic structure with three double bonds. Further examination of the ^1H NMR spectrum revealed that the olefinic proton at δ 5.53 (1H, *br d*, $J = 9.8$ Hz) coupled with another one at 5.51 (1H, *dt*, $J_1 = 9.8$ Hz, $J_2 = J_3 = 3.1$ Hz), which itself coupled with the methylene group CH_2-3 (δ 2.73, 1H, *ddd*, $J_1 = 19.9$ Hz, $J_2 = 3.15$ Hz, $J_3 = 1.6$ Hz; δ 2.90, 1H, *ddddd*, $J_1 = 19.9$ Hz, $J_2 = 5.4$ Hz, $J_3 = J_4 = 2.6$ Hz, $J_5 = 1.3$ Hz).

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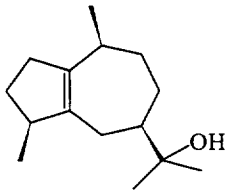

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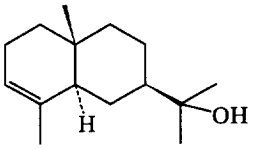

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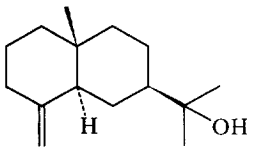

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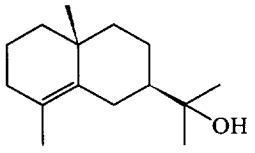

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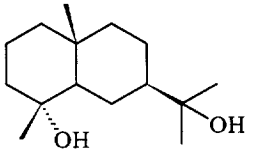

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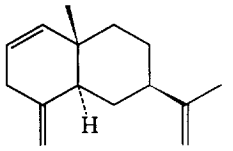

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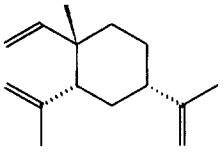

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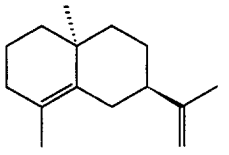

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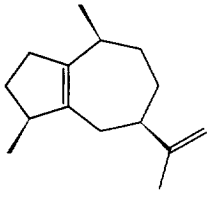

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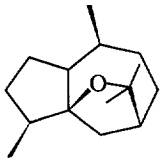

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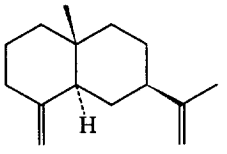

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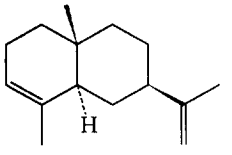

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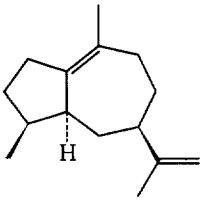

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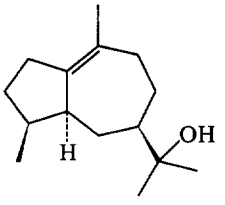

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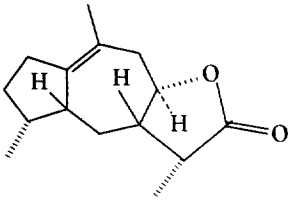

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Moreover, the ^1H–^1H COSY diagram showed coupling correlations between CH_2-3 and the olefinic protons at δ 4.59 and 4.84, leading to substructure -CH=CH-CH_2-C=CH_2-. The methine proton H-5 coupled with the methylene group CH_2-6 (δ 1.40, 1H, *dd*, $J_1 = 12.3$ Hz, $J_2 = 3.8$ Hz; δ 1.68, 1H, *br d*, $J = 12.3$ Hz) which itself coupled to the methine proton H-7 (δ 2.01, 1H, *tt*, $J_1 = J_2 = 12.3$ Hz, $J_3 = J_4 = 3.8$ Hz). H-7 further coupled to the methylene group CH_2-8 (δ 1.48, 1H, *dd*, $J_1 = 12.3$ Hz, $J_2 = 3.7$ Hz; δ 1.62, 1H, *m*), which was connected to another methylene group CH_2-9 (δ 1.43, 1H, *dd*, $J_1 = 12.3$ Hz, $J_2 = 3.6$ Hz; δ 1.60, 1H, *m*), giving the substructure -CH-CH_2-CH-CH_2-CH_2-. The combination of all these substructures permitted us to suggest an eudesmane skeleton for the compound. The relative stereochemistry at the positions C-5 and C-7 resulted from the coupling constant ($J = 12.3$ Hz for both of them) showing that

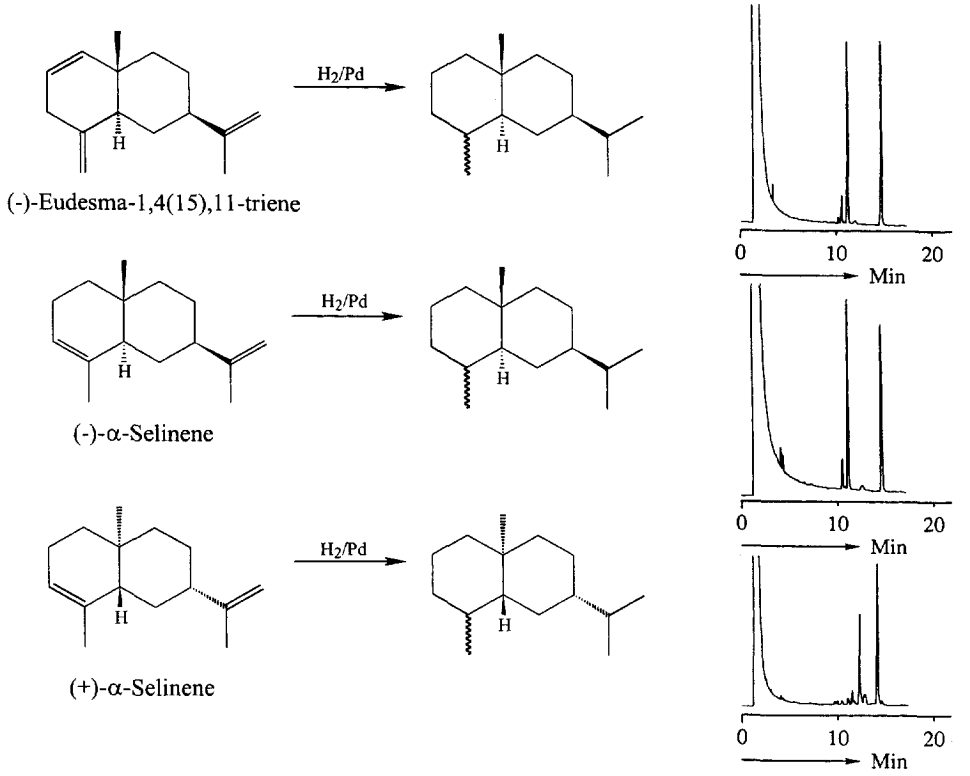

Fig. 1. Catalytic hydrogenation of eudesma-1,4(15),11-triene (**6**), (-)- and (+)-α-selinene and gas chromatographic comparison on a 25 m fused silica capillary column with heptakis(6-O-*t*.butyldimethylsilyl-2,3-di-O-methyl)-β-cyclodextrin (50% in OV 1701, w/w) at 120°C. The diastereoisomeric hydrogenation products of **6** and of (-)-α-selinene have the retention times 11.1 and 14.6 min, respectively; the hydrogenation products of (+)-α-selinene elute at 12.3 and 14.1 min.

H-5 and H-7 were in axial position. Moreover, the NOESY spectrum showed spatial interaction between H-5 and H-7 and also between the protons CH_3-14 and H-6a. To confirm this result, compound **6** was hydrogenated. Co-injection of the mixture of two diastereoisomeric hydrogenation products with a fully hydrogenated sample of (-)-α-selinene (**12**), also consisting of two diastereoisomers, using a cyclodextrin derived chiral stationary phase (Fig. 1), proved the identity and confirmed the spectroscopic result that all chiral centres (C-5, C-7 and C-10) of compound **6** had the same configuration as those of (-)-**12**, while a co-injection with a hydrogenated sample of (+)-α-selinene resulted in different retention times for both diastereoisomers.

EXPERIMENTAL

Essential oil

The essential oil of *Callitris intratropica* was a commercial product from Mr. K.-D. Protzen, Paul Kaders GmbH, Hamburg, and originates from South Africa.

GC/GCMS

The GC analyses were conducted on an Orion Analytica Micromat instrument equipped with two fused silica capillary columns in parallel (CPSil 5 CB, 25 m and CPSil 19 CB, 25 m). The operating temperatures for the detector and injector were 250° and 200°, respectively, while the oven temp. was programmed from 50° to 230° at 3° min^{-1}. The carrier gas was H_2 at 0.5 bar.

The GC-MS analyses were conducted on a Hewlett-Packard HP 5890A gas chromatograph with a CPSil 5 fused silica capillary column coupled to a VG Analytical 70–250 double-focusing mass spectrometer with an ion source temperature of 230°. The oven temperature was programmed from 80° to 270° at 10°C min^{-1}. Helium was used as a carrier gas.

Isolation methods

The essential oil (1 g) was subjected to low temp. liquid column chromatography at −25° in a 30 × 2.5 cm glass column packed with 40 g of silica gel (60–200 μm). The eluting solvents were *n*-pentane and EtOAC. One of the pentane fractions contained compounds **6**, **7** and **9** as a mixture while compounds **10** and **15** were in separate EtAOC fractions as a mixture. These three fractions were subjected to prep. GC on a Varian 1400 GC instrument equipped with a stainless steel column (Silcosteel, Amchro, 2.05 m × 5.1 mm, SE-30) at injector and detector temperatures of 200°

and 250°, respectively, while the column temperature was 125°. He was the carrier gas at a flow rate of 120 ml min^{-1}.

NMR spectroscopy

The isolated compounds were dissolved in $CDCl_3$ with TMS as the internal standard. The NMR techniques applied to compound **6** included ^1H NMR, ^1H-^1H COSY, ^{13}C, ^{13}C-^1H-COSY, COLOC and NOESY.

(-)-Eudesma-1,4(15),11-triene (**6**): ^1H NMR ($CDCl_3$, 500 MHz); δ 0.84 (3H, *s*, H-14), 1.40 (1H, *dd*, J_1 = 12.9 Hz, J^2 = 3.8 Hz, H-6), 1.43 (1H, *dd*, J_1 = 12.3 Hz, J_2 = 3.6 Hz, H-9), 1.48 (1H, *dd*, J_1 = 12.3 Hz, J_2 = 3.7 Hz, H-8), 1.60 (1H, *m*, H-9), 1.62 (1H, *m*, H-8), 1.68 (1H, *brd*, J = 12.3 Hz, H-6), 1.76 (3H, *s*, H-13), 2.01 (1H, *tt*, J_1 = 19.9 Hz, J_2 = 3.1 Hz, J_3 = 1.6 Hz, H-3), 2.90 (1H, *ddddd*, J_1 = 19.9 Hz, J_2 = 5.4 Hz, J_3 = J_4 = 2.6 Hz, J_5 = 1.3 Hz, H-3), 4.59 (1H, *brd*, J = 1.6 Hz, H-15), 4.72 (1H, *brs*, H-12), 4.74 (1H, *brs*, H-12), 4.84 (1H, *brd*, J = 1.6 Hz, H-15), 5.51 (1H, *dt*, J_1 = 9.8 Hz, J_2 = J_3 = 3.1 Hz, H-2), 5.53 (1H, *brd*, J = 9.8 Hz). ^{13}C NMR ($CDCl_3$, 500 MHz); δ 19.39(*q*), 21.39(*q*), 26.84(*t*), 29.02(*t*), 35.72(*t*), 37.29(*s*), 38.80(*t*), 46.18(*d*), 48.40(*d*), 106.40(*t*), 108.70(*t*), 123.31(*d*), 139.72(*d*), 148.04(*s*), 150.99(*s*). Ms (EI, 70 eV), *m/z*: 202(15) M^+., 187(22), 173(21), 159(60), 145(49), 131(59), 119(54), 105(85), 91(100), 79(56), 67(29), 55(31), 41(67).

(-)-α-Guaioxide (**10**): ^1H NMR ($CDCl_3$, 500 MHz); δ 0.91 (3H, *d*, J = 6.3 Hz), 0.98 (3H, *d*, J = 6.3 Hz), 1.15 (3H, *s*), 1.20 (1H, *m*), 1.26 (1H, *m*), 1.31 (3H, *s*), 1.35 (1H, *ddd*, J_1 = 3.5 Hz, J_2 = 7.6 Hz, J_3 = 13.6 Hz), 1.39 (1H, *m*), 1.43 (2H, *m*), 1.68 (1H, *dd*, J_1 = 6.9 Hz, J_2 = 4.1 Hz), 1.71 (1H, *m*), 1.75 (1H, *m*), 1.84 (1H, *m*), 1.88 (2H, *m*), 1.95 (2H, *m*). ^{13}C NMR ($CDCl_3$, 500 MHz); δ 15.14(*q*), 22.18(*q*), 22.31(*q*), 27.79(*t*), 29.38(*t*), 29.43(*q*), 30.45(*t*), 33.04(*t*), 36.42(*d*), 40.06(*t*), 44.14(*d*), 45.02(*d*), 54.74(*d*), 80.52(*s*), 90.57(*s*).

Dihydrocolumellarin (**15**): ^1H NMR (C_6D_6, 400 MHz); δ 0.47 (1H, *dd*, J_1 = 12.0 Hz, J_2 = 23.4 Hz, H-6), 0.86 (3H, *d*, J = 6.6 Hz, Me-15), 0.93 (1H, *dd*, J_1 = 4.1 Hz, J_2 = 11.7 Hz, H-3), 1.01 (3H, *d*, J = 6.9 Hz, Me-13), 1.23 (1H, *m*, H-7), 1.26 (1H, *m*, H-4), 1.43 (1H, *m*, H-5), 1.49 (1H, *d*, J = 11.4 Hz, H-6), 1.52 (3H, *br s*, Me-14), 1.62 (1H, *m*, H-3), 1.71 (1H, *ddd*, J_1 = 6.8 Hz, J_2 = 13.2 Hz, J_3 = 20.3 Hz, H-11), 1.93 (1H, *m*, H-2), 2.20 (2H, *m*, H-2 and H-9), 2.38 (1H, *dd*, J_1 = 2.5 Hz, J_2 = 14.2 Hz, H-9), 3.30 (1H, *m*, H-8). ^{13}C-NMR (C_6D_6, 400 MHz); 12.88(*q*), 19.18(*q*), 23.79(*q*), 32.70(*t*), 32.86(*t*), 34.15(*t*), 40.39(*t*), 42.76(*d*), 43.27(*d*), 51.62(*d*), 55.20(*d*), 81.02(*d*), 122.96(*s*), 143.62(*s*), 177.53(*s*).

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REFERENCES

1. Dallimore, W. and Jackson, A. B., *Handbook of Coniferae*, Edward Arnold & Co, London, 1923, p. 165.
2. Baker, R. and Smith, H., *A Research on Pines of Australia*, W. A. Gullick & Co, Sydney, 1910, p. 172.
3. Rudman, P., *Chemistry and Industry*, 1964, **0**, 808.
4. Brecknell, D. J. and Carman, R. M., *Australian Journal of Chemistry*, 1979, **32**, 2455.
5. Hardt, I. and König, W. A., *Journal of Chromatography*, 1994, **A666**, 611.
6. Hirota, H., Tanahashi, Y. and Takahashi, T., *Bulletin of the Chemical Society of Japan*, 1980, **53**, 785.