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TWO GUAIANE AND EUDESMANE-TYPE SESQUITERPENOIDS FROM *NEOCALLITROPSIS PANCHERI*

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Key Word Index—*Neocallitropsis pancheri*; Cupressaceae; eudesmane and guaiane-type sesquiterpenoids; pancherione; eudesm-4(14)-ene-3α,11-diol; trans-dihydrocarissone; α-costol; β-costol; γ-costol; 2D NMR.

Abstract—Two new sesquiterpenoids with the guaiane and eudesmane carbon skeleton termed pancherione and eudesm-4(14)-en-3α, 11-diol, were isolated from the heartwood of *Neocallitropsis pancheri* and characterized by 1D and 2D NMR spectroscopy. *Trans*-dihydrocarissone and α-, β- and γ-costols were also obtained from this plant for the first time and their NMR parameters are given.

INTRODUCTION

The heartwood of *Neocallitropsis pancheri Florin* [1] is a rich source of sesquiterpenoids [2]. While eudesmane-type compounds are the major constituents [2], four new sesquiterpene derivatives, with bisabolane, acorane and prezizane carbon skeletons, have been reported to occur in this oil [3–5]. The present work deals with the isolation and structural determination of two further new sesquiterpenoids, i.e. pancherione (**1**) and eudesm-4(14)-ene-3α,11-diol (**2**). Their structures were established as 1-azulenone-2,3,4,5,6,7-hexahydro-5-(1′-hydroxy-1′-methylethyl)-3,8-dimethyl for **1** and 2-naphthalene-methanol, decahydro-7-hydroxy-α,α,4a-trimethyl-8-methylene for **2** on the basis of 1D and 2D NMR spectral data. We report here also the occurence of *trans*-dihydrocarissone (**3**), and α-(**4**), β-(**5**) and γ-costol (**6**) for the first time in this plant.

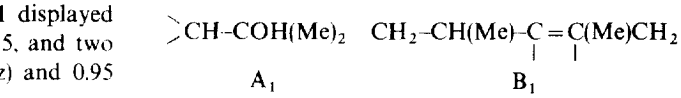
RESULTS AND DISCUSSION

The various distilled fractions of *N. pancheri* heartwood oil, when chromatographed on a silver nitrate argented column, afforded pancherione (**1**), eudesm-4(14)-ene-3α,11-diol (**2**), *trans*-dihydrocarissone (**3**), and α-(**4**), β-(**5**) and γ-costol (**6**).

The EIMS of **1** gave a $[M]^+$ peak at *m/z* 236 consistent with a molecular formula of $C_{15}H_{24}O_2$. The ^1H 400 MHz NMR spectrum of pancherione **1** displayed two methyl proton singlets at δ1.20 and 1.15, and two methyl proton doublets at δ1.07 (^3J = 7 Hz) and 0.95 (^3J = 7 Hz). The presence of a conjugated unsaturated carbonyl unit was evident from its IR spectrum (1721, 1648 cm^{-1}) and ^{13}C NMR data which also indicated two quaternary olefinic centres (δ177.9 and 145.1) and one ketone function (δ208.5). Furthermore, the IR spectrum showed a hydroxyl group band (3643 cm^{-1}). The DEPT pulse sequence was indicative of three methines, four methylenes and one oxygen-bearing quaternary aliphatic carbon. The above data indicated that **1** was a bicyclic sesquiterpenoid.

The structure of pancherione (**1**) and its ^1H and ^{13}C NMR spectral parameters were deduced from the concerted application of homonuclear and both direct and long-range heteronuclear chemical shift correlation experiments. Firstly, the establishment of the proton connectivities was deduced from the COSY spectrum [6, 7]. Then, one bond proton–carbon chemical shift correlations were achieved using a proton detected C,H-correlation experiment (HMQC sequence) [8], while assignments of the CH_n groups were obtained from analysis of the long-range correlation responses over two or three bonds (^2J or ^3J couplings) using the HMBC technique [9].

Beginning from the connectivities observed between the methyl proton shifts and the carbons α and β to these groups in the HMBC diagram, the two following partial structures A_1 and B_1 were determined:



A_1 B_1

Expansion to include other groups in structural segments A_1 and B_1 became feasible using (i) the proton

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intercoupling network obtained from the COSY diagram, (ii) the long-range ^1H–^{13}C correlation responses observed for the ^1H resonances other than methyls, (iii) chemical shift assignments (e.g. the deshielding of ^1H signals sited in α-position of the double bond function), and (iv) the previously reported 1D NMR results. From these data (Table 1), the structure of pancherione (**1**) was deduced to be 1-azulenone-2,3,4,5,6,7-hexahydro-5-(1′-hydroxy-1′-methylethyl)-3,8-dimethyl.

The molecular formula for **2** was established as $C_{15}H_{24}O_2$ (EIMS: $[M]^+ = m/z$ 236). Its IR spectrum showed the presence of a hydroxyl group (3646 cm^{-1}) and a C=C double bond (1645 cm^{-1}). The ^1H NMR spectrum indicated three methyl groups resonating as singlets (δ1.17 and 0.65), an exocyclic methylene group (δ4.57 and 4.91) and a proton linked to an oxygen-bearing carbon (δ4.26, *t*, J = 2.8 Hz). These results and the ^{13}C NMR data suggested a hydroxylated β-eudesmol skeleton for **2**. The location of the second alcohol function was determined on the basis of the COSY spectrum; the hydroxyl position was further supported by the correlation peak observed between the H-3 resonance and the vinylic protons (H_2-14). The suggested stereochemistry at C-3 (OH axial and α-) followed from the coupling constant pattern (see Experimental).

Finally, the molecular framework and the complete ^1H and ^{13}C chemical shift assignments of **2** were deduced (Table 2), as for pancherione (**1**), on the basis of the concerted application of homonuclear and heteronuclear chemical shift experiments. Consideration of the various connectivities in conjunction with the inferences drawn from the conventional spectrum established that **2** was eudesm-4(14)-ene-3α, 11-diol.

These results were consistent with those concerning some dihydroxylated eudesmane-type sesquiterpenoids such as the cryptomeridiol epimers from *Amanoa oblongifolia* [10].

Table 1. ^1H and ^{13}C NMR chemical shifts for **1**

δ_C*	Group†	Assignment‡	δ_H*
208.5	C	2	—
178.0	C	5	—
145.1	C	1	—
73.2	C	11	—
49.7	CH	7	1.29
43.0	CH_2	3	2.52 and 1.90
37.9	CH	4	2.67
32.8	CH_2	9	1.73 and 1.42
32.0	CH_2	6	2.64 and 2.22
27.9	Me	12	1.20
27.1	CH	10	2.88
26.6	CH_2	8	1.85 and 1.52
25.7	Me	13	1.15
19.3	Me	14	1.07
17.8	Me	15	0.93

* In ppm with respect to TMS.
† Determined from DEPT spectra.
‡ Information obtained from 2D experiments (COSY and HMBC).

Table 2. ^1H and ^{13}C NMR chemical shifts for **2**

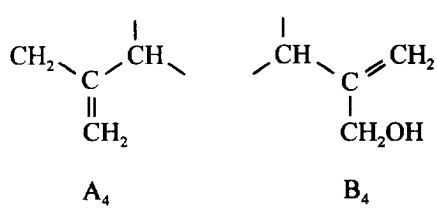
δ_C	Group†	Assignment‡	δ_H*,§
152.1	C	4	—
109.0	CH_2	14	4.91 and 4.57
73.6	CH	3	4.26
73.0	C	11	—
49.4	CH	7	1.38
43.6	CH	5	1.27
40.8	CH_2	9	1.48 and 1.25
35.8	C	10	—
35.8	CH_2	1	1.66 and 1.20
29.8	CH_2	2	1.75
27.3	Me	12	1.17
27.0	Me	13	1.17
24.6	CH_2	6	1.58 and 1.08
22.5	CH_2	8	1.60 and 1.27
15.6	Me	15	0.65

* In ppm with respect to TMS.
† Determined from DEPT spectra.
‡ Information obtained from 2D experiments (COSY and HMBC).
§ Determined from the cross-sections of heteronuclear chemical shift correlation diagram (HMQC).

The proposed configurations of **1** and **2** are based on biogenetic considerations and ^{13}C and ^1H chemical shifts comparisons with model compounds such as guaiol and β-eudesmol [12], for which the absolute configuration is well-documented.

Compound **3** was identified as *trans*-dihydrocarissone by comparing its EIMS spectra, and ^1H and ^{13}C NMR chemical shift data to the known compound [11]. However, the reported ^{13}C values for C-1 and C-9 were reversed on the basis of selective proton decoupling experiments.

The ^1H NMR data of **4** established the presence of two exocyclic methylene groups (δ5.02, 4.90, 4.68 and 4.40, each (br) 1H singlet), a tertiary methyl group (δ0.70) and an oxygen-bearing methylene resonance (δ4.12, *s*(br), 2H). The IR spectrum exhibited a hydroxyl group band at 3647 cm^{-1}. The presence of these functions was confirmed by the ^{13}C NMR spectrum. Additional analysis of the carbon multiplicities, determined from the DEPT pulse sequence, established the formula $C_{15}H_{24}O$. From the concerted use of HMQC and COSY 2D diagrams, the following partial substructures A_4 and B_4 were obtained:



A_4 B_4

At this point, **4** was indentified as α-costol on the basis of above arguments, and the similarity of its ^{13}C NMR spectrum with that of previously reported α-eudesmol [12]. Moreover, good agreement was observed between the ^{1}H resonances of **4** with the literature data [13].

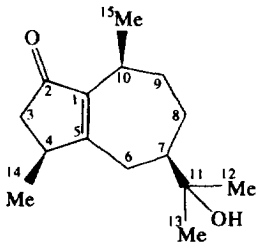
The ^{13}C NMR data for **5** and **6** suggested that these compounds were also eudesmanes, which were subsequently identified as β- and γ-costol, respectively. It should be noted that this is the first report of the ^{13}C data of costol isomers. It is noteworthy to remark that *N. pancheri* heartwood oil was essentially composed of various eudesmane-type sesquiterpenoids.

EXPERIMENTAL

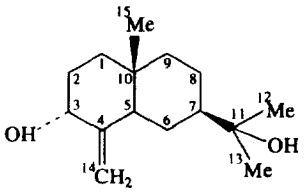
The heavy fraction of *N. pancheri* oil was obtained by distillation of the crude essential oil under red. pres. (vaccum: 0.4 mm Hg). This heavy fr. was chromatographed over a silica gel column using hexane to remove the hydrocarbon fr. followed by CH_2Cl_2 to give the polar fr. This last fr., which contained mainly sesquiterpenoids, was separated by CC on silica gel coated with $AgNO_3$ using a stepwise gradient system from toluene to EtOAc [4]. Compounds **1**–**6** were obtained from the following frs: toluene–EtOAc (17:3) gave **6**, (4:1) gave **5**, (7:3) gave **3**, (3:2) gave **4**, (9:11) gave **1**, and (7:13) gave **2**. Repeated elution, where necessary, was used purify every compound. 1D and 2D NMR: $CDCl_3$ with TMS as int. stand., ^{1}H at 400.13 MHz and ^{13}C at 100.61 MHz. Standard Bruker pulse sequences were used for homonuclear and heteronuclear correlation experiments (DEPT, HMBC, HMQC, COSY). For other experimental details see refs [14, 15].

Pancherione (**1**). $C_{15}H_{24}O_2$; Oil, $[\alpha]_D^{25}$ − 21° ($CHCl_3$; *c* 0.1); IR ν_{max} cm^{-1}: 3643, 2970, 2883, 1721, 1648, 1384; EIMS 70 eV, *m/z* (rel. int.): 236 $[M]^+$ (1), 218 (25), 178 (34), 163 (42), 105 (12), 91 (20), 59 (100); ^{1}H NMR ($CDCl_3$): δ0.93 (3H, *s*, H-15), 1.07 (3H, *d*, J = 7.1 Hz, H-14), 1.15 (3H, *s*, H-13), 1.20 (3H, *s*, H-12), 1.29 (1H, *ddt*, ^{1}J = 12.0 Hz, ^{2}J = 10.2 Hz, ^{3}J = 2.0 Hz, H-7), 1.42 (1H, *ddt*, ^{1}J = 11.5 Hz, ^{2}J = 11.0 Hz, ^{3}J = 3.2 Hz, H-9),, 1.52 (1H, *m*, H-8), 1.73 (1H, *m*, H-9), 1.85 (1H, *m*, H-8), 1.90 (1H, *dt*, ^{1}J = 18.16 Hz, ^{2}J = 1.3 Hz, H-3), 2.22 (1H, *dd* (*br*), ^{1}J = 15.7 Hz, ^{2}J = 11.7 Hz, H-6), 2.52 (1H, *dd*, ^{1}J = 18.6 Hz, ^{2}J = 6.5 Hz, H-3), 2.64 (1H, *dt*, ^{1}J = 15.1 Hz, ^{2}J = 1.8 Hz, H-6), 2.67 (1H, *m*, H-4), 2.88 (1H, *ddq*, ^{1}J = 10.9 Hz, ^{2}J = 3.8 Hz, ^{3}J = 7.4 Hz, H-10).

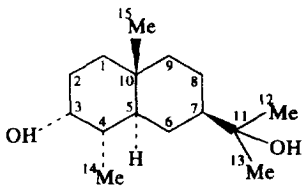
Eudesm-4(14)-ene-3α, 11-diol (**2**). $C_{15}H_{24}O_2$; Oil,$[\alpha]_D^{25}$ +20° 2 ($CHCl_3$; *c* 0.21); IR ν_{max} cm^{-1}: 3646, 1645, 1453, 1388, 1049, 905; EIMS 70 eV, *m/z* (rel. int.): 236 $[M]^+$ (1), 220 (3), 187 (8), 181 (12), 162 (20), 147 (45), 133 (15), 119 (12), 105 (23), 91 (21), 59 (100); ^{1}H NMR ($CDCl_3$): δ0.65 (3H, *s*, H-15), 1.17 (6H, *s*, H-12 and H-13), 2.27 (1H, *dm*, J = 11.2 Hz, H-5), 4.26 (1H, *t*, J = 2.8 Hz, H-3), 4.57 (1H, *t*, J = 1.7 Hz, H-14), 4.91 (1H, *t*, J = 1.4 Hz, H-14).



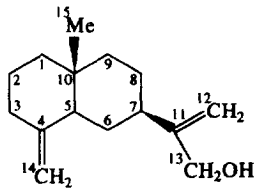
1 Pancherione



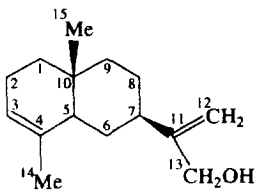
2 Eudesm-4 (14)-ene-3α, 11-diol



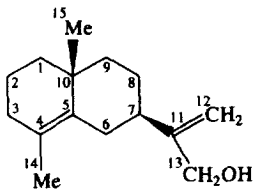
3 *Trans*-dihydrocarissone



4 α-Costol



5 β-Costol



6 γ-Costol

Trans-*dihydrocarissone* (**3**). $C_{15}H_{26}O_2$; IR ν_{max} cm^{-1}: 3580, 1703, 1466, 1384, 1151, 1133, 1088, 907; EIMS 70 eV, *m/z* (rel. int.): 238 $[M]^+$ (0), 223 (1), 180 (16), 125 (12), 123 (15), 99 (12), 95 (11), 83 (10), 81 (18), 79 (11), 77 (10), 69 (14), 67 (18), 55 (31), 53 (13), 43 (25), 41 (40), 39 (16), 59 (100); ^{13}C NMR ($CDCl_3$): δ213.2 (C-3), 72.7 (C-11), 51.1 (CH-5), 48.8 (CH-7), 45.7 (CH-4), 41.3 (CH_2-1), 40.8 (CH_2-9), 33.4 (C-10), 27.6 (Me-12), 26.9 (Me-13), 26.4 (CH_2-6), 22.1 (CH_2-8), 16.4 (Me-15), 11.3 (Me-14).

α-*Costol* (**4**). $C_{15}H_{24}O$; IR ν_{max} cm^{-1}: 3647, 1642, 1455, 1391, 1051, 907; ^1H NMR ($CDCl_3$): δ0.70 (3H, *s*, H-15), 1.22 (1H, *m*, H-9), 1.26 (1H, *m*, H-6), 1.28 (1H, *m*, H-1), 1.42 (1H, *m*, H-6), 1.44 (1H, *m*, H-8), 1.48 (1H, *m*, H-9), 1.55 (1H, *m*, H-2), 1.56 (1H, *m*, H-8), 1.57 (1H, *m*, H-1), 1.79 (1H, *m*, H-5), 1.97 (1H, *m*, H-3), 2.02 (1H, *m*, H-7), 2.27 (1H, *m*, H-3), 4.12 (2H, *s*(*br*), H-13), 4.40 (1H, *s*(*br*), H-14), 4.68 (1H, *s*(*br*), H-14), 4.90 (1H, *s*(*br*), H-12), 5.02 (1H, *s*(br), H-12); ^{13}C NMR ($CDCl_3$): δ154.2 (C-4), 107.2 (CH_2-12), 104.9 (CH_2-14), 65.1 (CH_2-13), 50.0 (CH-5), 41.9 (CH_2-1), 41.6 (CH-7), 41.2 (CH_2-9), 36.9 (CH_2-3), 36.1 (C-10), 30.0 (CH_2-6), 27.3 (CH_2-8), 23.5 (CH_2-2), 16.3 (Me_3-15).

β-*Costol* (**5**). $C_{15}H_{24}O$; ^{13}C NMR ($CDCl_3$): δ154.5 (C-11), 134.9 (C-4), 121.1 (CH-3), 107.3 (CH_2-12), 65.1 (CH_2-13), 46.9 (CH-5), 42.4 (CH-7), 40.3 (CH_2-9), 37.9 (CH_2-1), 32.3 (C-10), 29.4 (CH_2-6), 27.5 (CH_2-8), 23.0 (CH_2-2), 21.2 (Me-14), 15.7 (Me-15).

γ-*Costol* (**6**). $C_{15}H_{24}O$; ^{13}C NMR ($CDCl_3$): δ154.2 (C-11), 134.6 (C-5), 125.0 (C-4), 107.6 (CH_2-12), 65.3 (CH_2-13), 42.7 (CH-7), 42.3 (CH_2-1), 40.3 (CH_2-9), 34.6 (C-10), 33.2 (CH_2-3), 31.2 (CH_2-6), 28.3 (CH_2-8), 24.7 (Me-15), 19.4 (Me-14), 19.1 (CH_2-2).

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