

TRITERPENES FROM THE RESIN OF *BOSWELLIA NEGLECTA*

Aman Dekebo¹, Ermias Dagne^{1,*}, Odd R. Gautun² and Arne J. Aasen³

¹Department of Chemistry, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia

²Department of Chemistry, University of Tromsø, 9037 Tromsø, Norway

³School of Pharmacy, University of Oslo, P.O. Box 1068, Blindern, 0316 Oslo, Norway

(Received March 13, 2002; revised May 17, 2002)

ABSTRACT. The resin of *Boswellia neglecta* yielded four triterpenes canaric acid, α -amyrin, α -amyrone and *epi*- α -amyrin. Canaric acid and *epi*- α -amyrin are isolated here for the first time from the family Burseraceae. The compounds were identified using 1D and 2D NMR techniques.

KEY WORDS: *Boswellia neglecta*, Canaric acid, α -Amyrin, α -Amyrone, *epi*- α -Amyrin

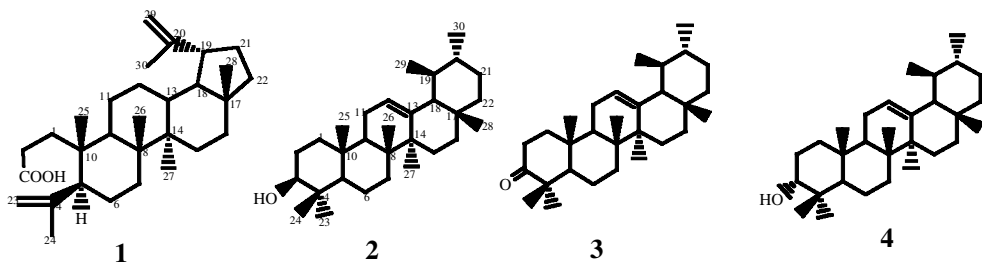
INTRODUCTION

Boswellia neglecta S. Moore (Burseraceae) is found in Bale, Gamo Gofa, Hararghe and Sidamo (Ethiopia) and also in neighbouring countries such as Kenya, Somalia, Tanzania and Uganda [1]. The plant produces aromatic resins used widely as incense known as "Dakara" (Oromifa), "Borena Etan" (Amharic) or "Borena type olibanum". Black or white resins of *B. neglecta* exude out from the trunk during the dry seasons. The white resin gradually comes out of the black gum during hot seasons. In general more black gum is produced than white, and the latter which is believed to be more aromatic is the one that is more costly. The resin is used by local people for the treatment of skin diseases and as repellent against insects, snakes and rats.

A previous report [2] on the volatile oils of several resin samples of *B. neglecta* indicated that α -thujene, α -pinene, *p*-cymene and limonene are the major components. Here we describe isolation and identification of the triterpenes, canaric acid, α -amyrin, α -amyrone and *epi*- α -amyrin from the olibanum of *B. neglecta*.

RESULTS AND DISCUSSION

The petrol extract of *B. neglecta* when chromatographed on silica gel using increasing amounts of EtOAc in *n*-hexane yielded four known triterpenes, canaric acid (1), α -amyrin (2), α -amyrone (3) and *epi*- α -amyrin (4).



*Corresponding author. E-mail: eda@telecom.net.et

Compound **1**, obtained as white crystals, was optically active and showed IR absorption band at 1706 cm^{-1} indicating the presence of a carboxyl group. EIMS and NMR spectral measurements indicated the compound has a 3,4-secolupene structure. Consequently, **1** was identified as canaric acid, previously reported from the oleoresin of *Canarium mulleri* [3, 4]. However, these workers characterized the compound on the basis of incomplete spectral data. ^{13}C NMR assignment of compound **1** was established using HSQC, HMBC and COLOC (Fig. 1). Furthermore, the known compounds, α -amyrin (**2**) and α -amyrone (**3**) were readily identified by comparison of their spectral data with a literature report [5]. α -Amyrin (**2**) was previously isolated from the resin of *B. serrata* (Burseraceae) [6, 7].

Compound **4** is a white powder exhibited an IR absorption band at 3450 cm^{-1} due to the presence of a hydroxyl moiety. ^{13}C NMR data of **4** exhibited a signal at $\delta\ 76.2\ d$ for C-3, which is upfield shifted, compared to (*ca.* $\delta\ 78.2\ \text{ppm}$), for those compounds with 3β -hydroxy configuration. Based on the evidences above and comparison of the ^{13}C NMR data and ^1H NMR data with similar compounds in the literature [7] **4** was identified as *epi*- α -amyrin.

To the best of our knowledge this is the first report of **1** and **4** from the family Burseraceae and there was no previous ^{13}C NMR data. Fig. 2 shows some important HMBC of compound **4**.

Table 1. ^{13}C NMR spectral data of compounds **1-4** in CDCl_3 .

C No.	1	2	3	4
1	33.9	38.8	39.6	33.3
2	24.6 ^a	27.5	26.6	26.6
3	180.6	79.0	217.2	76.2
4	147.6	38.8	47.5	37.4
5	50.4	55.3	55.3	48.9
6	28.4 ^a	18.4	19.7	18.4
7	32.8	33.0	34.3	32.8
8	40.6	40.0	40.1	40.2
9	40.8	47.8	46.9	47.6
10	39.3	36.9	36.7	37.0
11	21.5	23.4	23.6	23.3
12	25.1	124.3	124.2	124.5
13	38.0	139.6	139.7	139.6
14	43.3	42.2	42.3	42.2
15	27.5	28.2	28.1	28.2
16	35.6	26.7	26.6	25.3
17	43.1	33.8	33.8	33.8
18	48.3	59.1	59.2	59.2
19	48.1	39.7	39.7	39.7
20	150.9	39.7	39.7	39.7
21	29.9	31.3	31.3	31.3
22	40.0	41.6	41.6	41.6
23	113.5	28.1	28.8	28.8
24	23.3	15.7	21.5	22.4
25	20.2	15.7	15.5	15.5
26	16.1	16.9	16.9	16.9
27	14.6	23.3	23.2	23.4
28	18.1	28.1	26.6	28.4
29	109.6	17.5	17.5	17.5
30	19.4	21.5	21.4	21.4

Signal assignments with the same superscripts in the same column may be interchangeable.

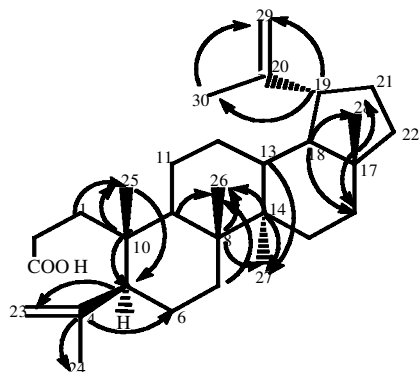


Fig.1 COLOC of compound **1**.
Arrows point from C to H.

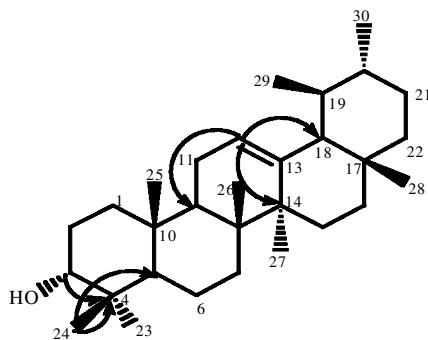


Fig. 2 Selected HMBC of **4**.
Arrows point from H to C.

EXPERIMENTAL

General. Melting points are uncorrected. TLC was performed on precoated plates (Silica gel 60 F₂₅₄, Merck) using *n*-hexane/EtOAc (9:1) as eluent and with vanillin-H₂SO₄ as detecting reagent. CC was performed on silica gel. IR spectra were measured on a Perkin Elmer 1600 using KBr. ¹H and ¹³C NMR were recorded on a JEOL JNM-EX400 instrument at 400 MHz and 100 MHz, respectively, using CDCl₃ as solvent and internal reference. EIMS data were obtained on VG Quattro quadrupole mass spectrometer operated at 70 eV.

Plant material. Resins and botanical specimens of *B. neglecta*, were collected from trees growing near Dibluk, Sidamo, Ethiopia in December, 1996. Locally *B. neglecta* tree is known as “Dakara”. The plant was identified by Dr. Kaj Vollesen, Kew Botanic Gardens, and a specimen with voucher number 072813 has been deposited at the National Herbarium of Addis Ababa University, Ethiopia.

Extraction and isolation of triterpenes. The pulverized resin (100 g) was soaked in petrol for one day at room temperature and then concentrated to give a yellowish oil (36 g, 36%). The extract (15 g) was chromatographed on silica gel (220 g) using increasing amounts of EtOAc in *n*-hexane as eluents. Further purification of the fractions by CC and CPTLC yielded compounds **1** (240 mg), **2** (450 mg), **3** (125 mg) and **4** (25 mg).

Canaric acid (I). White crystals (CHCl₃), m.p. 212–214 °C, R_f: 0.20, [α]_D²²: +38° (c. 0.3, CHCl₃). UV λ_{max}^{EtOH} nm (log ε): 206 (3.47), IR ν_{max}^{KBr} cm⁻¹: 3072, 2943, 1706, 1453, 1381, 1305, 1224, 888, 760; EIMS *m/z* (rel. int.): 440 [M]⁺ (55), 426 (6), 425 (14), 367 (24), 360 (28), 359 (100), 329 (26), 291 (12), 275 (15), 257 (9), 218 (17), 195 (17), 175 (13), 163 (15), 149 (22), 147 (20), 121 (41), 119 (26); ¹H NMR δ: 4.83 (1H, *br s*, H-23a), 4.68 (1H, *d*, *J* = 1.8 Hz, H-29a), 4.64 (1H, *br s*, H-23b), 4.57 (1H, *br s*, H-29b), 1.72 (3H, *s*, Me-24), 1.68 (3H, *s*, Me-30), 1.06 (3H, *s*, Me-27), 0.95 (3H, *s*, Me-28), 0.83 (3H, *s*, Me-25); 0.78 (3H, *s*, Me-26); ¹³C NMR: Table 1.

α -Amyrin (2). White crystals from hexane, m.p. 178-180 °C; R_f: 0.21, IR ν_{\max}^{KBr} cm⁻¹: 3294, 2940, 1459, 1383, 1189, 1037, 994; EIMS *m/z* (rel. int.): 426 [M]⁺ (4), 408 (18), 393 (4), 365 (6), 218 (100), 203 (37), 190 (16), 189 (36), 175 (31), 161 (28), 147 (36), 145 (29), 135 (41), 134 (32), 133 (48), 121 (64), 120 (24), 119 (64), 109 (37), 105 (65), 93 (67), 81 (58), 69 (69), 55 (95), 41 (79); ¹H NMR δ : 5.16 (1H, *t*, *J* = 3.3 Hz, H-12), 3.21 (1H, *dd*, *J* = 5.84, 4.76 Hz, H-3), 0.77-0.99 (21H, *m*, 7 x Me) and 1.06 (3H, *s*, Me); ¹³C NMR: Table 1.

α -Amyrone (3). Amorphous, R_f: 0.52, IR ν_{\max}^{KBr} cm⁻¹: 3010, 2960, 1700, 1450, 1380, 1110, 980, 750; EIMS *m/z* (rel. int.): 424 [M]⁺ (28), 409 (9), 314 (1), 218 (100), 203 (15), 189 (6.2), 149 (5), 133 (7), 123 (6), 95 (7), 55 (7); ¹H NMR δ : 5.14 (1H, *t*, *J* = 3.64 Hz, H-12), 0.78-1.06 (21H, *m*, 7 x Me) and 1.08 (3H, *s*, Me); ¹³C NMR: Table 1.

epi- α -Amyrin (4). White crystals (hexane), m.p. 103-105°C, R_f: 0.38, $[\alpha]_D^{22}$: +53.3°, IR ν_{\max}^{KBr} cm⁻¹: 3450, 3010, 2940, 2860, 1450, 1380, 1070, 990, 730, 760; EIMS *m/z* (rel. int.): 426 [M]⁺ (15), 219 (17), 218 (100), 203 (13), 190 (9), 189 (10), 135 (7), 121 (4), 95 (5), 93 (3), 69 (4); ¹H NMR δ : 5.12 (1H, *t*, *J* = 3.5, H-12), 3.39 (1H, *br s*, H-3), 0.78-0.99 (21H, *m*, 7 x Me) and 1.07 (3H, *s*, Me); ¹³C NMR: Table 1.

ACKNOWLEDGEMENTS

This work is supported by a grant from Sida-SAREC (Sweden). AD is grateful to Gondar College of Medical Sciences and DAAD-NAPRECA for a fellowship.

REFERENCES

1. Vollesen, K. in Vol 3, *Flora of Ethiopia*, Hedberg, I.; Edwards, S., (Eds.); National Herbarium, Science Faculty, Addis Ababa University, Addis Ababa, **1989**, p 442.
2. Provan, G.J.; Gray, A.I.; Waterman, P.G. *Flavour Fragr. J.* **1987**, *2*, 115.
3. Carman, R.M.; Cowley, D.E. *Tetrahedron Lett.* **1964**, 627.
4. Carman, R.M., Cowley, D. *Aust. J. Chem.* **1965**, *18*, 213.
5. Lau-Cam, C.A. *Phytochemistry* **1973**, *12*, 475.
6. Pardhy, R.S., Bhattacharyya, S.C. *Indian J. Chem.* **1978**, *16B*, 171.
7. Mahajan, B., Taneja, S.C., Sethi, V.K., Dhar, K.L. *Phytochemistry* **1995**, *39*, 453.