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CYTOTOXIC ABIETANE DITERPENOIDS FROM *CARYOPTERIS INCANA*

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Key Word Index—*Caryopteris incana*; Verbenaceae; abietane diterpenoid; incanone; cytotoxicity.

Abstract—A new rearranged abietane, incanone, together with a known abietane diterpenoid, sugiol, were isolated from the whole plant of *Caryopteris incana*. On the basis of the HRMS- and NMR data incanone was determined as 11,12,14,16-tetrahydroxyl-17(15 → 16)-*abeo*-abieta-8,11,13-trien-one. Incanone showed cytotoxic activity against human leukaemia cells. Copyright © 1997 Elsevier Science Ltd

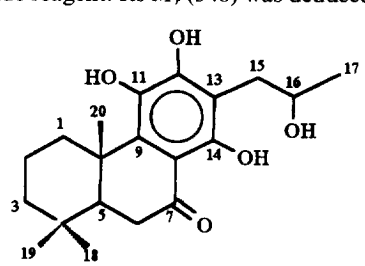
INTRODUCTION

The whole plant of *Caryopteris incana* (Thunb.) Miq. has been used in China as a folk medicine for the relief of colds, coughs and rheumatic pains [1]. Up to now, there is no report about its chemical constituents except for one on the volatile oil [2]. In this paper, we report on the isolation and structural elucidation of a new rearranged abietane, incanone, together with a known abietane diterpenoid, sugiol, from the title plant.

RESULTS AND DISCUSSION

Compounds **1** and **2** were isolated from the EtOAc-soluble fraction of a 95% ethanol extract of *C. incana*. Compound **2** was identified as sugiol by comparing its spectral data (^{1}H and ^{13}C NMR, EI–MS, IR) with those in the literature [3].

Incanone (**1**) gave a green coloration on spraying with ferric chloride reagent. Its M_r (348) was deduced by EIMS and the molecular formula $C_{20}H_{28}O_5$ was determined by HRMS (*m/z* 348.1932; calcd. 348.1936). The ^{1}H NMR spectrum of **1** revealed the presence of four methyl groups [δ 0.94 (3H, *s*, H-18), 0.96 (3H, *s*, H-19), 1.18 (3H, *d*, J=6.3 Hz, H-17) and 1.37 (3H, *s*, H-20)], two pairs of doublet doublets between δ 2.49 and 2.89 corresponding to two methylene groups, and a multiplet at δ 4.17 (1H) attributed to a CH group linked to an oxygen. The ^{13}C NMR spectrum and a DEPT experiment (Table 1) indicated the presence of a ketone group [δ 204.9 (C)], a hexa-substituted aromatic ring and one oxygenated carbon [δ 69.6 (CH)], as well as signals of four methyl groups, five methylene groups, one methine group and two quartenary sp^3 subunits. The ^{1}H–^{1}H COSY and HMQC spectra established the following segments that were isolated by quarternary carbons: -CH_2CH_2 CH_2-, > $CHCH_2$-, -$CH_2CH(OH)CH_3$. The above results are consistent with a 17(15 → 16)-*abeo*-abietane diterpenoid [4–6]. The skeleton of incanone and the assembly of these subunits were determined from



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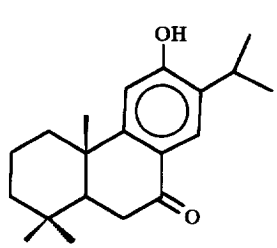

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detailed analysis of the HMBC spectrum of **1** (Fig. 1). HMBC correlation of δ_C 111.3 (C-13) to H-15 and H-16 and of δ_C 153.2 (C-14) and δ_C 158.1 (C-12) to H-15, suggested the connection of C-13 to C-15. Similarly, HMBC correlation of δ_C 204.9 (C-7) to δ_H 2.49

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Table 1. ^1H and ^{13}C NMR spectral data of compound **1** and ^{13}C NMR data of compound **2** (400 MHz and 100 MHz, CD_3COCD_3, ppm, J = Hz)*

H	1	C	1	DEPT	2
1β	3.42 *ddd*(13.2, 3.3, 2.2)	1	37.2	CH_2	38.6
1α	1.30 *ddd*(13.2, 13.2, 3.9)	2	19.6	CH_2	19.5
2	1.74 *m*	3	41.8	CH_2	42.1
	1.54 *m*	4	33.9	C	33.8
3	1.25 *m*	5	50.8	CH	50.5
	1.46 *m*	6	35.8	CH_2	36.4
5α	1.75 *dd*(15.0, 3.0)	7	204.9	C	197.3
6β	2.66 *dd*(17.1, 15.0)	8	109.2	C	124.3
6α	2.49 *dd*(17.1, 3.0)	9	136.5	C	156.9
15	2.89 *dd*(14.7, 2.1)	10	40.9	C	38.6
15′	2.73 *dd*(14.7, 7.2)	11	136.8	C	110.2
16	4.17 *m*	12	158.1	C	160.7
17	1.18 *d*(6.3)	13	111.3	C	133.6
18	0.94 *s*	14	153.2	C	126.4
19	0.96 *s*	15	31.7	CH_2	27.3
20	1.37 *s*	16	69.6	CH	22.7
		17	22.8	CH_3	22.6
		18	33.4	CH_3	32.8
		19	21.8	CH_3	21.6
		20	17.9	CH_3	23.4

* Assignment from ^1H–^1H COSY, HMQC, HMBC and NOESY.

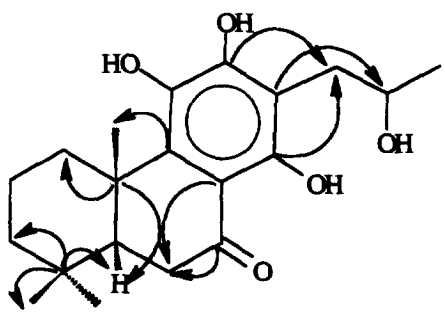

Fig. 1. 2D HMBC data for compound **1**. Correlation: $^{13}C \rightarrow {}^1H$.

(H-6α) and 2.66 (H-6β), and of 109.2 (C-8) to δ_H 2.49 (H-6α), suggested the connection of C-7 to C-6 and C-8 to C-7. The sp^3 quarternary carbon at δ_C 40.9 (C-10) showed a correlation with H-1β and H-5, whereas the carbon at δ_C 33.9 (C-4) showed correlation with H-5α and H-3, indicating the connection of C-10 to C-1 and C-5, C-4 to C-3 and C-5. These data led to the proposed structure of compound **1**.

The relative stereochemistry was deduced from the NOESY spectrum. **1** showed NOEs between Me-19 and Me-20, and between Me-18 and H-5α. No NOE was observed between Me-20 and H-5α for a *trans* relative disposition. Therefore, Me-20 was β, Me-18 was α-equatorial and Me-19 was β-axial.

In preliminary pharmacological tests, incanone showed significant cytotoxic activity against human leukaemia cells HL_{60} ($IC_{50} = 6 \times 10^{-6}$ M).

EXPERIMENTAL

^1H and ^{13}C NMR: 400 and 100 MHz, respectively, chemical shifts are given in δ relative to TMS as int. standard; 2D NMR: 500 MHz; TLC: silica gel 60 F_{254} (Merck) prepared plates developed with petrol-Me_2CO (3:1).

Extraction and isolation. Whole plants of *C. incana* (Thunb.) Miq. were collected at Zhang County, Gansu Province, P.R. China, in September 1993. A voucher specimen (BMU-930920) is deposited in the Department of Botany, School of Pharmaceutical Science, Beijing Medical University.

The air-dried and powdered whole plants (6 kg) were extracted with 95% EtOH (22 l × 2). The combined extracts were evapd. under red. pres. to dryness (680 g). The residue (565 g) was chromatographed over silica gel (500 g, 100–200 mesh) eluted with cyclohexane (7000 ml), EtOAc (7000 ml), BuOH (7000 ml) and MeOH (5000 ml). A portion (90 g) of the EtOAc eluate (100 g) was chromatographed over silica gel (200–300 mesh) with petrol (60–90°) followed by increasing concns of Me_2CO. Five frs were collected. Fr. 2 was chromatographed on a silica gel column eluting with CH_2Cl_2 and MeOH (20:1) to give compound **2** (10 mg, $R_f = 0.50$). Fr. 3 was chromatographed on a silica gel column eluting with petrol (60–90°)-Me_2CO (6:1) to yield compound **1** (80 mg, $R_f = 0.42$).

Incanone (**1**). Yellow needle crystals $[\alpha]_D^{25} + 140.8°$ ($CHCl_3$, $c = 0.1$). UV λ_{max}^{MeOH} nm (1gε): 362 (3.6), 286 (4.0), 247 (3.5); IR ν_{max}^{KBr} cm^{-1}: 3430, 3210, 2918, 2606, 1625 (acyl keto), 1600, 1538, 1446, 1368, 1323, 1268, 1251, 1214, 1139, 1116, 1092, 941, 915, 848; ^1H NMR and ^{13}C NMR: Table 1; EIMS *m/z* (rel. int.): 348 $[M]^+$ (52), 330 $[M-H_2O]^+$ (100), 315 $[M-H_2O-CH_3]^+$ (76), 304 (10), 273 (13), 245 (18), 179 (11), 69 (5).

Sugiol (**2**). Needles, mp 282–285°, $[\alpha]_D^{25} + 12.3°$

($CHCl_3$, $c = 0.1$). IR ν_{max}^{KBr} cm^{-1}: 3434, 2922, 2850, 1638 (acyl keto), 1595, 1459, 1371, 1308, 1216, 1177, 909; ^{13}C NMR: Table 1; EIMS *m/z* (rel. int.): 300 $[M]^+$ (94), 285 (100), 243 (30), 229 (12), 217 (47), 203 (34), 189 (13), 163 (23), 115 (16), 91 (11), 69 (23), 55 (18).

Cytotoxicity. The assay was dependent on the reduction of MTT by the mitochondrial dehydrogenase of viable cells to a blue formazan product which can be measured spectrophotometrically [7, 8].

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REFERENCES

1. Jiangsu New Medical College, *The Chinese Medicine Dictionary*. Shanghai People's Publishing House, Shanghai, 1977, p. 774.
2. Pu, Z. L., Shi, Y., Yang, Y. C., Zhang, J. and Lu, Y. C., *Acta Chimica Sinica*, 1984, **42**, 1103.
3. Jolad, S. D., Hoffmann, J. J., Schram, K. H. and Cole, J. R., *Journal of Natural Products*, 1984, **47**, 983.
4. Bruno, M., de la Torre, M. C., Savona, G., Piozza, F. and Rodriguez, B., *Phytochemistry*, 1990, **29**, 2710.
5. Alder, A. C., Rüedi, P., Prewo, R., Bieri, J. H. and Eugster, C. H., *Helvetica Chimica Acta*, 1986, **69**, 1395.
6. Rüedi, P., *Helvetica Chimica Acta*, 1986, **69**, 972.
7. Denizot, F. and Lang, R., *Journal of Immunological Methods*, 1986, **89**, 271.
8. Mosmann, T., *Journal of Immunological Methods*, 1983, **65**, 55.