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THREE FRAGRANT SESQUITERPENES OF AGARWOOD*

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Key Word Index—*Aquilaria* sp. (*A. malaccensis*); Thymelaeaceae; agarwood; fragrant sesquiterpenes; biogenetic precursor; (−)-10-epi-γ-eudesmol; α-agarofuran; oxo-agarospirol.

Abstract—Three fragrant sesquiterpenes have been isolated as major constituents from the wood of *Aquilaria malaccensis* and identified as α-agarofuran, (−)-10-epi-γ-eudesmol and oxo-agarospirol.

INTRODUCTION

Agarwood (Jinkoh in Japanese) is a widely used, famous incense of the Orient. About 20 years ago, Bhattacharyya *et al.* identified eight sesquiterpenes [1–4], including agarospirol [2], α- and β-agarofuran [3], from an agarwood (type A) originating from *Aquilaria agallocha* Roxb. and recently we have characterized four new companion sesquiterpenes, together with agarospirol, from the other kind (type B) of agarwood (*Aquilaria* sp.; probably *Aquilaria malaccensis* Benth.) [5, 6]. We report here the identification of three known fragrant sesquiterpenes, i.e. α-agarofuran (**1**), (−)-10-epi-γ-eudesmol (**2**) and oxo-agarospirol (**3**), newly isolated from type B agarwood.

RESULTS AND DISCUSSION

After column chromatography, preparative TLC and HPLC separation of the benzene extract, each of the sesquiterpenes was isolated in a pure form. The isolated ether, α-agarofuran (**1**), had the same optical rotation and IR spectrum (film) as those reported for authentic samples [3, 7], and its other spectral (^{1}H NMR and ^{13}C NMR, MS and accurate MS) data (see Experimental) were consistent with structure **1**. The second polar terpene, an alcohol, $C_{15}H_{26}O$, $[M]^+$ 222.2007, $[\alpha]_D$ −68.8°, was, on the basis of its IR (film), ^{1}H NMR (400 MHz; $CDCl_3$), accurate mass and CD spectral evidence (see Experimental), assigned structure **2**, and it was finally identified as (−)-10-epi-γ-eudesmol (**2**) by direct comparison of the IR (film) [8, 9], ^{1}H NMR ($CDCl_3$; 400 MHz [8] and 200 MHz [10]), mass [8, 10] spectra and optical rotation value with those of authentic standards [8–10]. The most polar terpene, a hydroxy-aldehyde, $C_{15}H_{24}O_2$, $[M - H_2O]^+$ 218.1669, $[\alpha]_D$ −18.5°, was identified as oxo-agarospirol (**3**) [11] by direct comparison with an authentic sample (see Experimental). Oxo-agarospirol was first isolated by us from a different kind of agarwood (Kanankoh in Japanese), which is a more expensive and precious type agarwood than the usual type (Jinkoh), and the structure was elucidated as **3**. These results have recently been presented in a preliminary form ¶ [11]. In the natural kingdom, (−)-10-epi-γ-eudesmol (**2**) has previously been found only in geranium [10] and vetiver [8] essential oils. However, the co-occurrence of α-agarofuran (**1**) and its postulated precursor (**2**) in nature was found for the first time. By analogy with vetiver sesquiterpene biogenesis [8], **2** is assumed to be the precursor of the nootkatane and spirovetivane derivatives [6] primarily occurring in type B agarwood.

EXPERIMENTAL

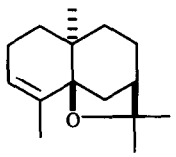
IR: liquid film; CD: in i-octane; ^{1}H NMR at 400 MHz in $CDCl_3$ with TMS as internal standard, unless otherwise noted, MS and accurate MS at 70 eV, optical rotations: in $CHCl_3$, unless otherwise stated The HPLC conditions were the same as described in our previous paper [6], except for the changes in column length (30 cm × 2), in eluant flow (2 ml/min) and in eluting solvents (see the isolation procedure). Kieselgel 60, silica gel HF-254 and PF-254 (Merck) were used for CC, TLC and prep. TLC, respectively.

Plant material. The agarwood used in this study was collected in Indonesia and imported via Singapore. The source plant belongs to the genus *Aquilaria* and has been tentatively identified by one of us (K.Y) as *Aquilaria malaccensis* Benth. The exact identification seems to be very difficult but is now under progress.

Extraction and isolation The C_6H_6 extract (130 g) was obtained from crushed wood (1.3 kg) as described previously [5, 6]. A portion (117 g) of the extract was fractionated on a silica gel (70–230 mesh; 1 kg) column with C_6H_6 as eluant. The elution was continued until oxo-agarospirol (**3**), one of the most polar sesquiterpene components in type-B wood, was entirely eluted. Both separated fractions, containing jinkohol (1 46 g) [5] and four major alcohols (agarospirol, jinkoh-eremol, kusunol and jinkohol II) (5.9 g) [6] were used for our previous work [5, 6]. All of the fractions, except the two mentioned above, were combined

*Part 3 in the series "Sesquiterpene Constituents in Agarwood". For Part 2, see ref. [6].

¶Recently, Chinese chemists have characterized an aldehyde named baimuxinal from Chinese agarwood [*Aquilaria sinensis* (Lour.) Gilg.] [12], and the structure might be the same as oxo-agarospiral (**3**)



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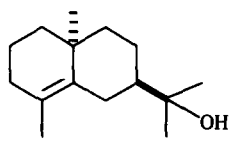

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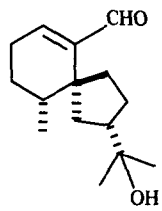

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and concd to yield an oily residue (10.1 g). Part (7.0 g) of the residue was subjected to prep. TLC (0.5 mm thickness; developed with C_6H_6–EtOAc, 10:1, detected by spraying water and by UV irradiation) to afford eight bands, i.e. bands I–VIII numbered in order of increasing polarity. Each separated band was eluted with $CHCl_3$ or $CHCl_3$–MeOH (20:1).

The oily component (200 mg) obtained from band I was purified further by HPLC (*n*-hexane–EtOAc, 15:1) to give α-agarofuran (**1**) (77 mg), a colourless oil, $[\alpha]_D$ +50.8° (*c* 0.60) [ref. [3] +37.09° (*c* 6.12); ref. [7] +41.5° (*c* 3.6)]. IR (film) as refs. [3, 7]; MS *m/z* (rel. int.): 220 $[M]^+$ (78.3), 205 (26.4), 202 (26.4), 187 (16.2), 147 (31.9), 123 (32.7), 109 (28.5), 105 (23.0), 91 (26.5), 82 (100), 81 (20.5), 55 (21.4); accurate MS *m/z*: $[M]^+$ 220.1832, $[M-Me]^+$ 205.1592, $[M-H_2O]^+$ 202.1722 and $[M-Me-H_2O]^+$ 187.1486 [calc. for $C_{15}H_{24}O$ (M) 220.1828, $C_{14}H_{21}O$ (M−Me) 205.1592, $C_{15}H_{22}$ (M−H_2O) 202.1722, $C_{14}H_{19}$ (M−Me−H_2O) 187.1486]; ^{1}H NMR: δ0.91 (3H, *s*, angular Me), 1.24 (3H, *s*), 1.37 (3H, *s*) (gem. dimethyl), 1.72 (3H, *br s*, olefinic Me), 2.22 (1H, *dd*, *J* = 4 and 12 Hz, allylic H), 5.62 (1H, *br s*, olefinic H) (cf. refs. [3, 7] 60 MHz; CCl_4); ^{13}C NMR (50 MHz, $CDCl_3$, TMS): δ19.1, 22.1, 22.8, 23.0, 24.8, 30.5, 32.7, 33.1, 34.7, 37.3, 44.8, 81.1, 85.2, 127.5 and 133.0.

The oily mixture (510 mg) obtained from band III was subjected to HPLC separation (*n*-hexane–EtOAc, 15:1) to afford (−)-10-epi-γ-eudesmol (**2**) (205 mg), a colourless oil, $[\alpha]_D$ −68.8° (*c* 1.02) [−70.0° ($CHCl_3$); Dr. P. Naegeli, personal communication]. IR (film) as ref. [9]; MS *m/z* (rel. int.): 222 $[M]^+$ (14.1), 204 (68.7), 189 (100), 161 (65.7), 133 (47.6), 91 (38.2), 59 (54.5); accurate MS *m/z*. $[M]^+$ 222.2007, $[M-H_2O]^+$ 204.1869 and $[C_3H_7O]^+$ 59.047 [calc. for $C_{15}H_{26}O$ (M) 222.1984, $C_{15}H_{24}$ (M−H_2O) 204.1877 and C_3H_7O (isopropanol moiety) 59.050]; ^{1}H NMR. δ1.09 (3H, *s*, angular Me), 1.19 (3H, *s*), 1.25 (3H, *s*), (gem. dimethyl), 1.68 (3H, *s*, olefinic Me), 2.12 (1H, *br d*, *J* = 15 Hz, allylic H), 2.72 (1H, *br d*, *J* = 15 Hz, allylic H) (cf. ref. [10] 200 MHz, $CDCl_3$; ref. [9] 60 MHz, CCl_4), CD (*c* 0.131): $[\theta]_{234}$ 0, $[\theta]_{215}$ +30 500, $[\theta]_{206}$ 0. The sign and molecular ellipticity of the CD Cotton curve (due to the isolated double bond) of the isolated alcohol (**2**) were compared with those of γ-eudesmol [CD (*c* 0.180): $[\theta]_{232}$ 0, $[\theta]_{216}$ −18 100, $[\theta]_{208}$ 0], and the absolute structure shown in **2** was inferred from this CD evidence and the other spectral data cited above.

Each syrupy residue obtained from bands VI (180 mg), VII (20 mg) and VIII (300 mg) was separated by HPLC (*n*-hexane–EtOAc, 5·1) and a total of 168 mg of oxo-agarospirol (**3**), a colourless oil, $[\alpha]_D^{25}$ −18.5° (*c* 0.0054, EtOH) [ref. [11] $[\alpha]_D^{25}$ −22.7° (*c* 0.0013, EtOH)] and $[\alpha]_D^{15}$ −4.9° (*c* 0.69, EtOH), was isolated. MS *m/z* (rel. int.): 218 $[M-H_2O]^+$ (37.5), 203 (30.1), 177 (100), 59 (50.3); accurate MS *m/z*: $[M-H_2O]^+$ 218. 1669, $[M-H_2O-Me]^+$ 203.1441 and $[M-C_3H_7O]^+$ 177.1273 [calc. for $C_{15}H_{22}O$ (M−H_2O) 218.1669, $C_{15}H_{19}O$ (M−H_2O−Me) 203.1436 and $C_{12}H_{17}O$ (M−isopropanol) 177.1273]. The isolated hydroxy-aldehyde (**3**) was identified by comparison with authentic oxo-agarospirol [11] [IR (film), ^{1}H NMR (90 MHz; CCl_4) and mass spectra and retention times (GLC)].

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REFERENCES

1. Jain, T. C. and Bhattacharyya, S. C. (1959) *Tetrahedron Letters* 13.
2. Varma, K. R., Maheshwari, M. L. and Bhattacharyya, S. C (1965) *Tetrahedron* **21**, 115.
3. Maheshwari, M. L., Jain, T. C., Bates, R. B. and Bhattacharyya, S. C. (1963) *Tetrahedron* **19**, 1079.
4. Maheshwari, M. L., Varma, K. R. and Bhattacharyya, S. C (1963) *Tetrahedron* **19**, 1519.
5. Nakanishi, T., Yamagata, E., Yoneda, K. and Miura, I. (1981) *Phytochemistry* **20**, 1597.
6. Nakanishi, T., Yamagata, E., Yoneda, K., Miura, I. and Mori, H. (1983) *J. Chem. Soc. Perkin Trans.* 1, 601.
7. Barrett, H. C. and Büchi, G. (1967) *J Am. Chem. Soc.* **89**, 5665.
8. Kaiser, R. and Naegeli, P. (1972) *Tetrahedron Letters* 2009.
9. Marshall, J. A. and Pike, M. T. (1968) *J. Org. Chem.* **33**, 435.
10. Pesnelle, P., Corbier, B. and Teisseire, P. (1970) *La France et ses Parfums* **12**, 412.
11. Nagashima, T., Kawasaki, I., Yoshida, T., Nakanishi, T., Yoneda, K. and Miura, I., 9th International Congress of Essential Oils, Singapore, 13–17 March 1983.
12. Yang, J.-S. and Chen, Y.-W. (1983) *Acta Pharmaceutica Sinica* **18**, 191.