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JINKOHOL, A PREZIZANE SESQUITERPENE ALCOHOL FROM AGARWOOD

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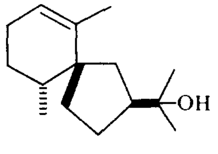
Key Word Index—*Aquillaria* sp.; agarwood; new sesquiterpene alcohol; new prezizane derivative; jinkohol; 2β-hydroxy-(+)-prezizane.

Abstract—A new tricyclic sesquiterpene, jinkohol, has been isolated from an agarwood (*Aquillaria* sp.) which is different from that obtained from *Aquillaria agallocha*. Its structure was shown to be 2β-hydroxy-(+)-prezizane by spectroscopic methods and by chemical transformation. The two kinds of agarwood are readily identified from their sesquiterpene components.

INTRODUCTION

Agarwood (Jinkoh in Japanese) is a widely used, famous incense of the Orient. In the course of chemical and morphological studies on agarwood, we noticed that two kinds of wood are popular on the market today. Preliminary experiments using GC/MS and/or TLC showed that they could be easily distinguished from each other because they contained different sesquiterpenoids. From one kind of agarwood, originating from *Aquillaria agallocha* Roxb., agarol [1], six selinanic furanoids represented by α- and β-agarofurane [2,3], and agarospirol (**1**) [4] have been isolated and characterized. The other kind of agarwood, collected in Indonesia and imported via Singapore, also contains considerable amounts of sesquiterpenoids. The structure of a predominant one, jinkohol (**2**), is the subject of this paper.



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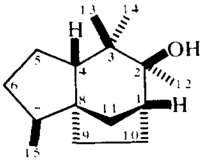

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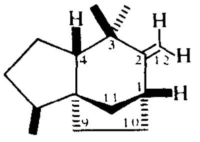

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RESULTS AND DISCUSSION

Fractionation of the benzene extract of agarwood by repeated column chromatography over silica gel afforded no agarospirol (**1**) but gave instead a new sesquiterpenoid alcohol, jinkohol.

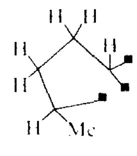
Jinkohol (**2**), colourless oil, $[\alpha]_D$ $-6.1°$ ($CHCl_3$), showed a hydroxy frequency (3610 cm^{-1}) in its IR spectrum. Its MS contained prominent ions at m/z 222.1987 (M^+, 99%) and 204.1869 ($M^+ - H_2O$, 42%) which were consistent with a molecular formula of $C_{15}H_{26}O$. Its proton noise-decoupled and off-resonance decoupled ^{13}C NMR spectra (100 MHz, $CDCl_3$) showed the presence of four methyl, five methylene, three methine and three quarternary carbons. These assignments suggested that **2** was a tricyclic sesquiterpene with a tertiary hydroxyl group. The assignments for the established structure, which were made with the aid of selective proton-decoupled ^{13}C NMR experiments, are listed in Table 1.

The ^1H NMR spectrum (400 MHz, $CDCl_3$) of **2** revealed the presence of a secondary methyl group, three tertiary methyl groups, and contained signals due to five methylenic and three methinic groups (Table 2). Double resonance experiments and ^{13}C NMR spectrometry using a selective proton-decoupling technique suggested four possible partial structures (**4a**–**4d**) for **2**. The presence of the five-membered ring structure **4b** was shown by the presence of two long-range couplings through four σ-bonds in a periplanar *W* arrangement ($J_{11\beta,9\beta} = J_{11\beta,10\beta} = 1.5$ Hz). When all the above-mentioned structural evidence was considered from a biogenetical viewpoint [6], then the most likely structure for jinkohol was that of the prezizane-type alcohol shown by formula **2**. On addition of $Eu(fod)_3$, two groups of protons, i.e. 1β-H and 2α-Me (i) and 4β- and 11β-Hs (ii), which were respectively vicinal and in a 1,3-diaxial relation to the axial 2β-OH, showed the largest and next largest downfield shifts, and all of the other protons were, more or less, moved

Table 1. ^{13}C NMR data of **2** (100 MHz, $CDCl_3$, TMS as internal standard)

Carbon No.*	δ	Carbon No.*	δ
1	50.1 (*d*)	9	21.1 (*t*)
2	77.1 (*s*)	10	26.4 (*t*)
3	52.5 (*s*)	11	38.1 (*t*)
4	55.7 (*d*)	12	24.0 (*q*)
5	21.8 (*t*)	13	27.1 (*q*)
6	31.1 (*t*)	14	21.1 (*q*)
7	39.2 (*d*)	15	14.4 (*q*)
8	40.0 (*s*)		

* Jinkohol (**2**) numbered with the accepted numbering system for fused rings [5].



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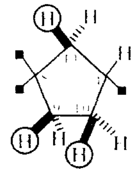

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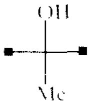

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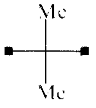

4d

downfield (Table 2), as was consistent with the assumed structure (**2**).

The absolute configuration of **2** was established as follows. Dehydration of **2** with $POCl_3$ in pyridine gave an alkene (**3**), the spectral data [IR (film), ^{1}H NMR (90 MHz, CCl_4), MS, and high resolution MS spectra] of which were identical with those published for natural (+)- and (−)-prezizaene [6, 7]. However, our sample showed an optical rotation of +10° ($CHCl_3$; *c* 0.60). Although we could not take its value close to that [+55° (MeOH; *c* 0.02)] reported for (+)-prezizaene [6], its positive sign suggested its absolute structure to be the same as that which is accepted for (+)-prezizaene (**3**). Since a direct comparison of samples under the same conditions was impossible because of the unavailability of **3**, the purity of our sample and the validity of the structure assigned to it was further corroborated by detailed ^{1}H NMR (400 MHz, $CDCl_3$) analysis. The observed spectrum demonstrated that our sample was pure. The assignments for the related protons were carried out by double resonance and NOE experiments as follows. The two proton signals (AB*q*) at δ 4.67 and 4.72 were ascribed to an exocyclic methylene at C-2. H-1 of **3** (δ 2.77, *dd*) exhibited a downfield shift by δ 0.76 compared with that of **2**; this was due to an anisotropic effect arising from the adjacent *exo*-methylene. The low field signal (δ 4.72) of the *exo*-methylene showed a NOE enhancement of 8 % on irradiation of H-1. The other half (δ 4.67) of the methylene signal was also enhanced (8 %) by NOE on irradiation of the adjacent gem.-dimethyl function (δ 1.07 and 1.08). Moreover, the axial H-11β of **2** resonated at lower field than that of **3** (δ 1.10, *ddd*), due to the presence of an axial 2β-OH group. These steric features and the positive signed optical rotation were consistent with the absolute structure shown in formula **3**, i.e. (+)-prezizaene. This meant that the original alcohol, jinkohol, was 2β-hydroxy-(+)-prezizane (**2**).

Table 2. ^{1}H NMR data of **2** (400 MHz, $CDCl_3$, TMS as internal standard)

Assigned proton	δ	Lanthanide shift
1-H	2.01 *dd* (*br*), $J_{1,10\alpha} = 4.9$, $J_{1,11\alpha} = 4.4$	−3.30
2α-Me	1.18 *s*	−3.17
3α-Me	0.90 *s*	−1.36
3β-Me	0.91 *s*	−2.06
4-H	1.69 *dd*, $J_{4,5\alpha} = J_{4,5\beta} = 9.8$	−2.25
5α-H	1.51 complex	−0.70
5β-H	1.52 complex	−0.85
6α-H	1.85 complex	−0.37
6β-H	1.12 complex	−0.59
7α-H	1.80 complex	−0.52
7β-Me	0.84 *d*, $J = 6.2$	−0.30
9α-H	1.25 complex	−1.02
9β-H	1.26 complex	−0.79
10α-H	1.66 complex	−1.44
10β-H	1.64 complex	−0.94
11α-H	1.38 *dd*, $J_{11\alpha,1} = 4.4$, $J_{11\alpha,11\beta} = 10.6$	−1.02
11β-H	1.56 *ddd*, $J_{11\beta,11\alpha} = 10.6$, $J_{11\beta,9\beta} = J_{11\beta,10\beta} = 1.5$	−2.85

Coupling constants in Hz.

Recently, four prezizane sesquiterpenes from *Eremophila georgei* have been shown to belong to the (−)-series [7] but none of them is identical with the enantiomer of jinkohol. Jinkohol, to our knowledge, is the second natural (+)-prezizane sesquiterpene to be isolated.

EXPERIMENTAL

General remarks. MS and high resolution MS: 75 eV. TLC: pre-coated Si gel plates (Merck 60 F-254); detection, 1 % $Ce(SO_4)_2$–10 % H_2SO_4.

Plant material. The agarwood used in this study was collected in Indonesia and imported via Singapore. The source plant was considered to belong to the genus *Aquillaria*. The exact identification of the plant is under investigation.

Isolation of jinkohol (**2**). Crushed wood (1.3 kg) was extracted with 6 l. C_6H_6 (× 3) for 3 hr under reflux. Concn of the combined C_6H_6 extracts gave an oily residue (130 g), a portion (117 g) of which was chromatographed over Si gel (70–230 mesh; 1 kg). Elution with C_6H_6 afforded fractions (1.46 g) containing mainly **2**. The fractions were combined and rechromatographed over Si gel (230–400 mesh; 80 g). Elution with *n*-hexane–Et_2O (15:2) gave **2** (510 mg), colourless oil, in a pure state (TLC and GC/MS). When left to stand in a refrigerator for a week, **2** gave colourless needles, mp 34° (uncorr.). $[\alpha]_D$ −6.1° (*c* 0.76); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3610 (OH); ^{13}C NMR: Table 1; ^{1}H NMR and induced shift experiment with $Eu(fod)_3$: Table 2; MS *m/z* (rel. int.): 222 [M$^+$] (99), 204 [M$^+$ − H_2O] (42), 179 (79), 137 (41), 109 (59), 95 (59), 82 (60), 79 (40), 71 (100); high resolution MS m/z: 222.1987 (M^+, calc. for $C_{15}H_{26}O$ 222.1984), 204.1869 ($M^+ - H_2O$, calc. for $C_{15}H_{24}$ 204.1877).

Dehydration of **2**. **2** (99 mg) was dissolved in dry pyridine (7 ml) and $POCl_3$ (1 ml) and allowed to stand at 20° for 10 days, when TLC showed that *ca* a third of the starting material had been transformed into the desired alkene (**3**). The mixture was poured into ice-H_2O and extracted with C_6H_6. After concn of the solvent, the residue (80 mg) was chromatographed on a column of neutral Al_2O_3 (Act Super I; 8 g) developed with *n*-hexane, C_6H_6, and C_6H_6–EtOAc (5:1). The *n*-hexane fraction contained pure **3** (TLC on $AgNO_3$-Si gel and ^1H NMR), colourless oil (24 mg). The C_6H_6 and C_6H_6–EtOAc (5:1) fractions contained unreacted **2** (50 mg).

3 had the following physical properties; $[\alpha]_D + 10°$ (c 0.60); IR ν_{max}^{film} cm^{-1}: 3080, 1785, 1631, 892; MS m/z (rel. int.): 204 $[M^+]$ (69), 189 (37), 175 (12), 161 (30), 133 (100), 119 (40), 108 (61), 91 (75); high resolution MS: M^+ m/z 204.1877 (calc. for $C_{15}H_{24}$ 204.1877); ^1H NMR (400 MHz, $CDCl_3$, TMS): δ 0.86 (3 H, *d*, $J = 6.5$ Hz, Me-7β), 1.07 (3 H, *s*, Me-3α or Me-3β), 1.08 (3 H, *s*, Me-3β or Me-3α), 1.10 (1 H, *ddd*, $J_{11\beta,11\alpha} = 10.5$ Hz, $J_{11\beta,9\beta} = J_{11\beta,10\beta} = 1.8$ Hz, H-11β), 1.65 (1 H, *dd*, $J_{11\alpha,11\beta} = 10.5$ Hz, $J_{11\alpha,1} = 4.3$ Hz, H-11α), 2.77 (1 H, *dd*, $J_{1,11\alpha} = 4.3$ Hz, $J_{1,10\alpha} = 6.5$ Hz, H-1), 4.67, 4.72 (2 H, AB*q*, $J = 1.7$ Hz, $\Delta\nu = 20$ Hz, H_2-12); IR (film), MS, and ^1H NMR (90 MHz, CCl_4) data are in agreement with those published for natural (+)- and (−)-prezizaene [6, 7].

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REFERENCES

1. Jain, T. C. and Bhattacharyya, S. C. (1959) *Tetrahedron Letters* 13.
2. Maheshwari, M. L., Jain, T. C., Bates, R. B. and Bhattacharyya, S. C. (1963) *Tetrahedron* **19**, 1079.
3. Maheshwari, M. L., Varma, K. R. and Bhattacharyya, S. C. (1963) *Tetrahedron* **19**, 1519.
4. Varma, K. R., Maheshwari, M. L. and Bhattacharyya, S. C. (1965) *Tetrahedron* **21**, 115.
5. Overton, K. H., (ed.) (1971) *Specialist Periodical Reports, Terpenoids and Steroids*, Vol. I, pp. 109. The Chemical Society, London.
6. Andersen, N. H. and Falcone, M. S. (1971) *Chem. Ind.* 62.
7. Carrol, P. J., Ghisalberti, E. L. and Ralph, D. E. (1976) *Phytochemistry* **15**, 777.