Available online at www.sciencedirect.com
SCIENCE DIRECT®
PERGAMON
Phytochemistry 62 (2003) 1213–1216
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Guaiane sesquiterpenes from *Amoora rohituka*

Rasheduzzaman Chowdhury, Choudhury M. Hasan, Mohammad A. Rashid*

Phytochemical Research Laboratory, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh

Received 14 March 2002; received in revised form 14 November 2002

Abstract

The petroleum ether extract of the stem bark of *Amoora rohituka* afforded two novel guaiane-derived sesquiterpenoids, 6β,7β-epoxyguai-4-en-3-one (**1**) and 6β,7β-epoxy-4β,5-dihydroxyguaiane (**2**). The structures of **1** and **2** were determined by extensive NMR and MS analyses and by comparison of their spectral data with related compounds. The relative stereochemistry of the asymmetric centers in **1** and **2**, except at C-5 of **2**, were determined by selective 1D-NOESY experiments.

Keywords: *Amoora rohituka*; Meliaceae; Guaiane sesquiterpenoids

1. Introduction

Amoora rohituka Wight & Arn. (Bengali name: Pithraj) is an evergreen tree that grows wild and planted in many districts of Bangladesh (Ghani, 1998). Various parts of the plant are used in Bengali traditional medicine because of their anticancer, antimicrobial, anti-inflammatory and hepatoprotective properties (Kirtikar and Basu, 1980). *Amoora* species have shown to contain several triterpenoids (Chatterjee et al., 1970) including limonoids (Mulholland and Naidoo, 1999), steroids, an alkaloid (Harmon et al., 1979), a chromone (noreugenin), three flavonoid glycosides (Jain and Srivastava, 1985) and straight-chain aliphatic compounds (Talukder and Howse, 2000). The antiviral and antibacterial properties of the isolated limonoid rohitukin (Connolly et al., 1976), cytotoxicity of amoorastatin, growth inhibitory effect of 12α-hydroxyamoorastatin against murine P388 lymphocytic leukaemia cell lines (Polonsky et al., 1979) and feeding deterrent activity against *Tribolium castaneum* (Coleoptera: Tenebrionidae) of some secondary metabolites (Talukder and Howse, 2000) have been documented earlier. This paper deals with the isolation and structure elucidation of two new guaiane sesquiterpenes (**1**, **2**) from the stem bark of *A. rohituka*.

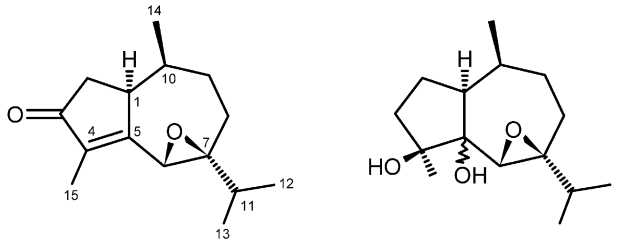
2. Results and discussion

The concentrated petroleum ether extract of *A. rohituka* was initially fractionated by vacuum liquid chromatography over Si gel. Purification of the early eluting VLC fractions by column chromatography followed by preparative TLC yielded two sesquiterpenes (**1**, **2**). Compound **1** was obtained as a colorless gum and its IR spectrum showed carbonyl (1703 cm^{-1}) absorption. The HRFABMS of **1** showed a pseudomolecular ion [M + H^+] at *m/z* 235.1695 which established its molecular formula as $C_{15}H_{22}O_2$, requiring five degrees of unsaturation. The ^{13}C NMR spectrum of **1** displayed 15 carbon resonances while the DEPT experiments indicated the presence of four methyls, three methylenes, four methines and four quarnernary carbons, including a ketone (δ_C 208.5), an oxygenated quaternary (δ_C 70.1) and a pair of sp^2 carbons (δ_C 141.1 and 167.9) assignable to a tetrasubstituted double bond. This suggested that compound **1** was tricyclic and comparison of these data with the guaiane sesquiterpenes, guai-6-en-10β-ol, and related compounds, sootepdienone (**3**) (Rukachaisirikul et al., 1998), orientalol C (**4**), and its analogs (Yoshikawa et al., 1992, Lago et al., 2000), suggested a guaiane type carbon skeleton for **1**.

The ^1H NMR spectrum of **1** displayed signals for an isopropyl moiety (methyl doublets at δ 1.00 and 1.07, and a methine multiplet at δ 1.68), a shielded methyl doublet at δ 0.53, and an olefinic methyl at δ 1.84. In

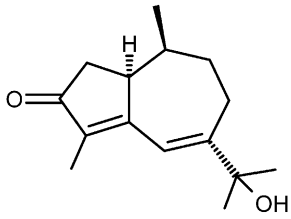
* Corresponding author. Tel.: +880-2-9661900-20x4798; fax: +880-2-8615583.
E-mail address: rashidma@aitlbd.net (M.A. Rashid).

0031-9422/03/$ - see front matter
doi:10.1016/S0031-9422(02)00698-2

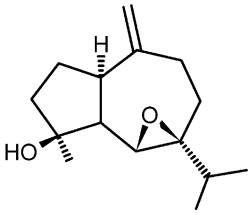


1

2



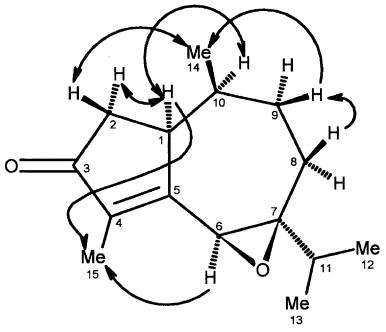
3



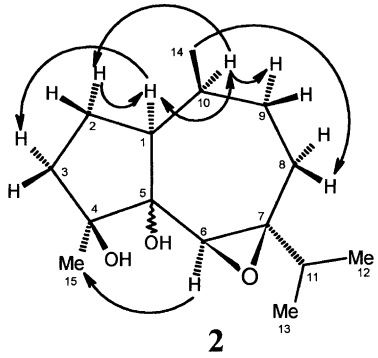
4

addition it also showed two methine proton resonances at δ 3.10 and 3.63, and three methylene protons at δ 2.50, 2.11, 1.97, 194, 1.87 and 1.58 (1H, each). The heteronuclear HSQC experiment confirmed that the proton at δ 3.63 was attached to a carbon bearing oxygen and the upfield shift of this carbon (δ_C 59.7) suggested its involvement in an epoxide ring formation. Treatment of **1** with acetic anhydride and pyridine did not give any acetyl derivative, which also confirmed the absence of any secondary hydroxyl group in this molecule. HMBC correlations from the isopropyl methyls to δ_C 70.1 (C-7) and 36.9 (C-11), and from H-11 (δ 1.68) to δ_C 70.1, 59.7 (C-6), and 20.9 (C-8) defined the locations of the isopropyl moiety and epoxide ring. The methyl at δ 1.84 showed HMBC correlations to δ_C 167.9 (C-5) and 141.1 (C-4) in addition to the carbonyl carbon at δ_C 208.5. The deshielded nature of C-5 could be explained by its β-position to the carbonyl group. On the other hand, HMBC correlations from the oxymethine proton at δ 3.63 (H-6) to δ_C 167.9, 141.1, 70.1 (C-7), 43.3 (C-1) and 36.9 (C-11) confirmed the double bond between C-4 and C-5. The methyl group at δ 0.53 revealed HMBC correlations to δ_C 43.3 (C-1), 32.8 (C-10) and 29.0 (C-9), while the C-2 methylene protons (δ 2.50 and 2.11) showed correlations over ^2J to 208.5 (C-3) and 43.3 (C-1) and over ^3J to δ_C 167.9 (C-5), 141.1 (C-4), and 32.8 (C-10). This established the planar structure of **1** to be 6β,7β-epoxyguai-4-en-3-one. The relative stereochemistry of chiral centers in **1** as depicted in Fig. 1 was determined by a series of selective 1D-NOESY interactions.

The molecular formula for compound **2** was established as $C_{15}H_{26}O_2$ from the FABMS and CIMS data. The ^{13}C NMR spectrum of **2** showed 15 signals, while DEPT and HMQC experiments confirmed that 12 out of the 15 carbons in **2** were attached to protons. The ^1H and ^{13}C NMR spectral data of **2** were, in part, identical to those observed for 6β,7β-epoxyguai-4-en-3-one (**1**), suggesting a close structural similarity between these two compounds. However, ^{13}C resonance appropriate for a carbonyl group in **1** was absent in the spectrum of **2**, and was replaced with resonances indicative of a methylene group. In fact, the ^{13}C NMR spectrum of **2** displayed signals for four methylene carbons, as compared with three in case of 6β,7β-epoxyguai-4-en-3-one (**1**). Another significant change in the ^{13}C NMR data between **1** and **2** was the absence of the olefinic carbons and the appearance of two additional oxymethine carbons (δ_C 69.5 and 69.1) in the latter compound. This suggested that compound **2** was the 3-deoxo-4,5-dihydroxy derivative of **1**. This was substantiated by the presence of strong absorption band at 3400 cm^{-1} in the IR spectrum of **2**. Compound **2** could not be acetylated with acetic anhydride and pyridine, which was also the case with structurally related orientalols (Yoshikawa et al., 1992, Lago et al., 2000), the ^1H NMR spectrum of the reaction mixture, though not separated, clearly showed signals assignable to compounds **1** and **2** in an approximate ratio of 1:1. This suggested that compound **2** could be converted to **1** by dehydroxylation with subsequent formation of the double bond between C-4 and



1



2

Fig. 1. Key NOE interactions observed in **1** and **2**.

C-5 and oxidation at C-3. The ^1H–^1H COSY and HMBC data observed with compound **2** were consistent with the proposed structure. Like compound **1**, the relative stereochemistry of the chiral centers in **2**, except at C-5, was also established by 1D-NOESY experiments (Fig. 1). Thus, compound **2** was identified as 6β,7β-epoxy-4β,5-dihydroxyguaiane.

Both compounds **1** and **2** were evaluated for their HIV-inhibitory (Gulakowski et al., 1991) and cytotoxic properties (Bokesch et al., 1999) in the U.S. National Cancer Institute's in-house assays but none showed any activities at 50 μg/ml. Compound **1** was tested against 19 bacterial strains by the standardized disc diffusion method (Bauer et al., 1966) but it only demonstrated mild in vitro antibacterial activity against *Escherichia coli* with a zone of inhibition of 10.5 mm at a dose of 50 μg/disc.

3. Experimental

3.1. General experimental procedures

The ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded in $CDCl_3$ on a Varian VXR 500S spectrometer and the chemical shifts are reported in ppm relative to the residual nondeuterated solvents. The number of attached protons for ^{13}C signals was determined using the DEPT pulse sequence. Inverse detected heteronuclear correlations were measured using the HSQC (optimized for $^1J_{CH} = 140$ Hz) and HMBC (optimized for $^nJ_{CH} = 8.3$ Hz) pulse sequences with a pulsed-field gradient. COSY-45 spectra were used to determine the proton-proton connectivities. Infrared (IR) spectra were obtained on a Perkin-Elmer 1600 FTIR spectrometer. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. CIMS was acquired in positive mode using *i*-butane as the reactant gas. HR-FABMS was recorded on a JEOL SX 102 mass spectrometer (resolving power = 10,000) using *m*-nitrobenzyl alcohol (NBA) or polyethylene glycol as matrix. Si gel 60 (70-230 mesh) was used for CC; TLC was carried out on Kieselgel PF_{254} plates (Merck), and the spots were visualized under UV (254 and 366 nm), and by spraying the plates with vanillin (1%)–H_2SO_4 (10%) in EtOH, followed by heating.

3.2. Plant material

The stem bark of *A. rohituka* was collected from Comilla district of southeastern Bangladesh in August 2000. The plant was identified by Professor M. Salar Khan, Ex-Research Consultant, Bangladesh National Herbarium. Voucher specimens for this collection have been deposited in the Bangladesh National Herbarium (DACB accession no. 28,927) and Dhaka University Herbarium (DUH accession no. 16).

3.3. Extraction and isolation of constituents

The air-dried and pulverized plant material (508 g) was extracted in a Soxhlet apparatus at elevated temperature using 2.5 l of light petroleum ether (40–60 °C). The extract was filtered and then evaporated under reduced pressure at 40 °C using a Büchi rotary evaporator to a gummy concentrate (6.6 g). An aliquot of the extract (4.6 g) was subjected to vacuum liquid chromatography over Si gel 60H, and the column was eluted with petroleum ether–EtOAc mixtures of increasing polarity, with nine fractions collected (each 50 ml). The VLC fraction-1 was further fractionated by column chromatography over Si gel 60 (70-230 mesh) using petroleum ether–EtOAc, EtOAc, EtOAc–MeOH mixtures of increasing polarity. Evaporation of solvents from fractions 19–22, followed by preparative TLC over Si gel PF_{254} using *n*-hexane–EtOAc (90:10) gave 6.4 mg of **2**, while similar treatment of fractions 28–32 using solvent system *n*-hexane–EtOAc (88:12) afforded **1** (10.1 mg).

3.4. Bioassays

DMSO solutions of both compounds **1** and **2** were assayed for cytotoxic properties in a 2-day in vitro assay (Bokesch et al., 1999) as well as for anti-HIV activities in an in vitro XTT-based assay, the experimental details of which have been reported previously (Gulakowski et al., 1991). Compound **1** was also tested against 19 bacterial strains by the standardized disc diffusion method (Bauer et al., 1966).

3.5. 6β,7β-Epoxyguai-4-en-3-one (*1*)

Colorless gum; $[\alpha]_D$ −10 ($CHCl_3$, *c* 0.5); IR (film) ν_{max} cm^{-1}: 2918, 2850, 1731, 1703, 1636, 1458, 1381; ^1H NMR: δ 0.53 (3H, *d*, *J* = 7.0 Hz, H-14), 1.00 (3H, *d*, *J* = 7.0 Hz, H-13), 1.07 (3H, *d*, *J* = 7.0 Hz, H-12), 1.58 (1H, *m*, H-9β), 1.68 (1H, *m*, H-11), 1.84 (3H, *d*, *J* = 1.5 Hz, H-15), 1.87 (1H, *m*, H-9α), 1.94 (1H, *m*, H-8β), 1.97 (1H, *m*, H-8α), 2.02 (1H, *m*, H-10), 2.11 (1H, *d*, *J* = 18.5 Hz, H-2β), 2.50 (1H, *dd*, *J* = 18.5, 7.0 Hz, H-2α), 3.10 (1H, *m*, H-1), 3.63 (1H, *bs*, H-6); ^{13}C NMR: δ 7.9 (C-15), 10.2 (C-14), 18.11 (C-13), 18.14 (C-12), 20.9 (C-8), 29.0 (C-9), 32.8 (C-10), 36.9 (C-11), 40.5 (C-2), 43.3 (C-1), 59.7 (C-6), 70.1 (C-7), 141.1 (C-4), 167.9 (C-5), 208.5 (C-3); HRFABMS: *m/z* $[M + H]^+$ 235.1695 (calc. for $[C_{15}H_{22}O_2 + H]^+$, 235.1698).

3.6. 6β,7β-Epoxy-4β,5-dihydroxyguaiane (*2*)

Colorless liquid; $[\alpha]_D$ −15 ($CHCl_3$, *c* 0.1); IR (film) ν_{max} cm^{-1}: 3400, 2924, 2853, 1458; ^1H NMR: δ 0.73 (3H, *d*, *J* = 6.5 Hz, H-14), 0.88 (3H, *d*, *J* = 7.0 Hz, H-13), 0.98 (3H, *d*, *J* = 6.5 Hz, H-12), 1.18 (1H, *dd*,

$J = 12.0$, 8.5 Hz, H-2α), 1.43 (3H, *s*, H-15), 1.45 (1H, *m*, H-11), 1.48 (1H, *m*, H-9α), 1.60 (1H, *m*, H-2β), 1.64 (1H, *m*, H-9β), 1.69 (1H, *m*, H-3α), 1.76 (1H, *m*, H-10), 1.85 (1H, *dd*, $J = 13.0$, 8.5 Hz, H-3β), 1.89 (1H, *ddd*, $J = 16.0$, 3.0, 3.0 Hz, H-8α), 1.98 (1H, *ddd*, $J = 16.0$, 13.0, 3.0 Hz, H-8β), 2.34 (1H, *dd*, $J = 8.5$, 3.0 Hz, H-1), 2.54 (1H, *bs*, H-6); ^{13}C: δ 12.6 (C-14), 15.2 (C-15), 18.1 (C-13), 18.2 (C-12), 21.1 (C-8), 26.4 (C-2), 29.0 (C-9), 33.2 (C-3), 33.9 (C-10), 36.9 (C-11), 45.7 (C-1), 64.3 (C-6), 66.2 (C-7), 69.1 (C-5), 69.5 (C-4); CIMS : m/z $[M + H]^+$ 255 (appropriate for $[C_{15}H_{26}O_3 + H]^+$); FABMS: m/z $[M + Na]^+$ 277.

Acknowledgements

We wish to thank the Molecular Targets Drug Discovery Program, National Cancer Institute at Frederick, MD 21702, USA for assisting with the NMR studies and bioassays and Dr. L.K. Pannell, Laboratory of Structural Mass Spectrometry, NIDDK, Bethesda, Maryland, USA for mass spectral measurements.

References

Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Turck, M., 1966. Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol. 45, 493–496.

Bokesch, H.R., Blunt, J.W., Westergaard, C.K., Cardellina II., J.H., Johnson, J.R., Michael, J.A., McKee, T.C., Hollingshed, M.G., Boyd, M.R., 1999. Alertenone, a dimer of suberosenone from *Alertigorgia* sp. J. Nat. Prod. 62, 633–635.

Chatterjee, A., Kundu, A.B., Chakrabortty, T., Chandrasekharan, S., 1970. Extractives of *Aphanamixis polystachya* wall. Structures and stereochemistry of aphanamixin and aphanamixinin. Tetrahedron 26, 1859–1867.

Connolly, J.D., Okorie, D.A., De Wit, L.D., Taylor, D.A.H., 1976. Structure of dregeanin and rohitukin, limonoids from the subfamily Melioideae of the family Meliaceae. An unusually high absorption frequency for a six-membered lactone ring. J. Chem. Soc., Chem. Commun. 22, 909–910.

Ghani, A., 1998. Medicinal Plants of Bangladesh: Chemical Constituents and Uses; Asiatic Society of Bangladesh, pp 84-85.

Gulakowski, R.J., McMahon, J.B., Staley, P.G., Moran, R.A., Boyd, M.R., 1991. A semiautomated multiparameter approach for anti-HIV drug screening. J. Virol. Methods 33, 87–100.

Harmon, A.D., Weiss, U., Silverton, J.V., 1979. The structure of rohitukine, the main alkaloid of *Amoora rohituka*. Tetrahedron Lett. 8, 721–724.

Jain, S.A., Srivastava, S.K., 1985. 8-C-methyl-quercetin-3-O–D-xylopyranoside, a new flavone glycoside from the roots of *Amoora rohituka*. J. Nat. Prod. 48, 299–301.

Kirtikar, K.R., Basu, B.D., 1980. Indian Medicinal Plants, 2nd ed. Published by Singh, B., Singh, M.P, India.

Lago, J.H.G., Brochini, C.B., Roque, N.F., 2000. Terpenes from leaves of *Guarea macrophylla* (Meliaceae). Phytochemistry 55, 727–731 (and references cited therein).

Mulholland, D.A., Naidoo, N., 1999. Limonoids from *Aphanamixis polystachya*. Phytochemistry 51, 927–930.

Polonsky, J., Varon, Z., Marazano, C., Arnoux, B., Pettit, G.R., Schmid, J.M., Ochi, M., Kotsuki, H., 1979. The structure of amoorastatone and the cytotoxic limonoid 12-hydroxyamoorastatin. Experientia 35, 987–989 (and references cited therein).

Rukachaisirikul, V., Naovanit, S.-A., Taylor, W.C., Bubb, W.A., Dampawan, P., 1998. A sesquiterpene from *Gardenia sootepensis*. Phytochemistry 48, 197–200.

Talukder, F.A., Howse, P.E., 2000. Isolation of secondary plant compounds from *Aphanamixis polystachya* as feeding deterrents against adult *Tribolium castaneum* (Coleoptera: Tenebrionidae). Z. Pflanzenkrankh. Pflanzenschutz 107, 498–504.

Yoshikawa, M., Hatakeyama, S., Tanaka, N., Fukuda, Y., Murakami, N., Yamahara, J., 1992. Orientalols A, B, and C, sesquiterpene constituents from Chinese Alismatis rhizoma, and revised structures of alismol and alismoxide. Chem. Pharm. Bull. 40, 2582–2584.