[BIO35] Synthesis of several bisabolane sesquiterpenoids from xanthorrhizol isolated from *C. xanthorrhiza* and their bioactivities

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Introduction

Xanthorrhizol (**1**) is a bisabolane-type sesquiterpenoid obtained as the main constituent of the rhizome oil of temu lawak, *Curcuma xanthorrhiza*, which has been utilised as a tonic in Indonesia and as a choleretic drug in Europe. As regards to its bioactivities, it has been shown that xanthorrhizol (**1**) possessed antifungal activity against *Candida albicans* (MIC=68.75 μg/mL) and cytotoxic against human nasopharyngeal carcinoma cell line (KB, EC_{50} = 4.90 μg/mL) (Mata et al., 2001). Although the synthesis of **1** has been extensively studied, the chemistry of this compound has only be studied in a limited extent. Thus, it is of great interest to explore the chemistry of **1** in order to exploit this substance as a starting material for conversion of other compounds. We noted that **1** can be converted to several bisabolane-type sesquiterpenoids, namely (10*R*/10*S*)-dihydro-10,11- dihydroxyxanthor-rhizols (**2**,**3**) and helibisabonol A diacetate (**4**). Triols **2** and **3** were isolated as minor constituents from the Mexican medicinal plant, *Iostephane heterophylla* (Aguilar et al., 2001). Helibisabonol A (**5**) is an allelochemical isolated from the CH_2Cl_2 extracts of dried sunflower leaves (*Helianthus annuus* L. cv. Peredovick) (Macías et al., 2002). To date, there is no enantioselective synthesis is reported for triols **2** and **3**, while only a racemic form of helibisabonol A (**5**) has been synthesised (Macías et al., 2002a).

In this paper, we report the stereoselective syntheses of triols **2** and **3** as well as synthesis of helibisabonol A diacetate (**4**). The bioactivities of xanthorrhizol and its derivatives were also reported in this paper.

Materials and methods

General

Petroleum ether refers to boiling range 60-80 °C and was redistilled before use. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm Merck silica gel plates (60 F_{254}), and compounds were visualised with UV light and acidic *p*-anisaldehyde. Merck silica gel 60 (0.040-0.063 mm) was used for vacuum liquid chromatography and column chromatography was carried out using Merck silica gel 60 (0.063-0.200 mm). Mass spectral data were obtained from Kent Mass Spectrometry Service, UK. ^{1}H and ^{13}C NMR spectra (300 and 75 MHz respectively) were recorded on a Bruker Avance 300 Spectrometer using $CDCl_3$ as solvent. IR spectra were recorded on a Shimadzu 8000 or a Perkin-Elmer series 1600 spectrometers as thin film (NaCl windows) for liquid samples or KBr pellet for solid samples. Optical rotations were determined on a JASCO DIP-370 digital polarimeter. Melting points (uncorrected) were measured on a Leica Galen III melting points apparatus.

Isolation of xanthorrhizol

The fresh rhizomes of *C. xanthorrhiza* (3.35 kg) were chopped and hydrodistilled in an all

1

2 10-S
3 10-R

4 R = Ac
5 R = H
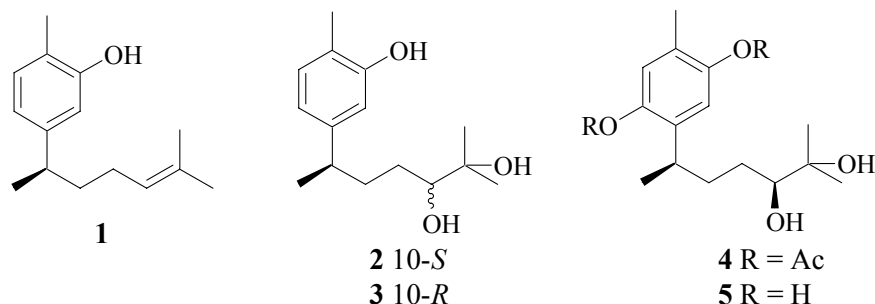
glass apparatus for 8 hours. The crude oil was extracted with diethyl ether (3 x 10mL), dried over anhydrous $MgSO_4$ and filtered. Evaporation of ether gave the essential oil (30.57 g, 0.91%) as a light yellow oil. The essential oil was purified by vacuum column chromatography to give **xanthorrhizol (1)** as a pale yellow oil (11.82g, 38.7%): $[\alpha]_D$ –49.4° (*c* 1.00, MeOH) {lit. [Rimpler et al., 1970] $[\alpha]_D$ –52.5° (CHCl₃)}; IR (neat) 3412, 2963, 1728, 1658, 1585, 1427, 1254, 1119, 993, 814 cm^{-1}; ^{1}H NMR δ 1.22 (3H, d, *J* 6.9 Hz, H-15), 1.55 (3H, s, H-12), 1.62 (2H, m, H-8), 1.69 (3H, s, H-13), 1.89 (2H, m, H-9), 2.24 (3H, s, H-14), 2.63 (1H, sextet, H-7), 4.69 (1H, s, OH), 5.11 (1H, br t, *J* 4.6 Hz, H-10), 6.63 (1H, d, *J* 1.8 Hz, H-2), 6.70 (1H, dd, *J* 7.8, 1.8 Hz, H-6), 7.05 (1H, d, *J* 7.8 Hz, H-5); ^{13}C NMR δ 15.3 (C-12), 17.7 (C-14), 22.4 (C-15), 25.7 (C-13), 26.1 (C-9), 38.4 (C-8), 39.0 (C-7), 113.5 (C-2), 119.4 (C-6), 120.8 (C-4), 124.5 (C-10), 130.3 (C-5), 131.4 (C-11), 147.2 (C-1), 153.6 (C-3); EIMS *m/z* 218 (35) [M$^+$, C₁₅H₂₂O], 136 (100), 121 (56).

Xanthorrhizyl acetate (6). To a stirred solution of xanthorrhizol (**1**) (1.00 g, 4.580 mmol) in diethyl ether (1 mL) was added acetic anhydride (0.935 g, 9.159 mmol) and pyridine (85 mg, 0.933 mmol). The reaction mixture was left to stir at rt for 73 h. The organic layer was washed with 5% HCl solution (3 x 5 mL) followed by saturated NaHCO₃ solution (2 x 5 mL) and dried over anhydrous $MgSO_4$. The solvent was evaporated to leave an oil which was chromatographed on silica gel (PE/Et₂O = 9/1) to give **xanthorrhizyl acetate (6)** (0.8453 g, 72.1%) as a colourless oil: R$_f$=0.45 (PE/Et₂O, 9/1); IR (neat) 2962, 1767, 1677, 1219 cm^{-1}; ^{1}H NMR (CDCl₃) δ 1.24 (3H, d, *J* 6.9 Hz, H-15), 1.53 (3H, s, H-12), 1.55-1.66 (2H, m, H-8), 1.69 (3H, s, H-13), 1.88-1.95 (2H, m, H-9), 2.16 (3H, s, H-14), 2.33 (3H, s, OAc), 2.69 (1H, sext, *J* 6.9 Hz, H-7), 5.10 (1H, tt, *J* 7.2, 1.2 Hz, H-10), 6.84 (1H, d, *J* 1.8 Hz, H-2), 6.99 (1H, dd, *J* 7.8, 1.8 Hz, H-6), 7.16 (1H, d, *J* 7.8 Hz, H-5); ^{13}C NMR δ 15.8 (C-14), 17.7 (C-12), 20.9 (C-17), 22.2 (C-15), 25.7 (C-13), 26.1 (C-9), 38.3 (C-8), 38.9 (C-7), 120.3 (C-2), 124.4 (C-6), 124.7 (C-4), 127.2 (C-10), 130.8 (C-5), 131.5 (C-11), 146.9 (C-1), 149.3 (C-3), 169.3 (C=O); EIMS *m/z* 260 (17) [M$^+$, C₁₇H₂₄O₂], 218 (10), 148 (38), 136 (100), 121 (32).

(7*R*,10*S*)-Acetoxydiol (7). A 25 mL round-bottomed flask, equipped with a magnetic stirrer, was charged with *tert*-butanol (5 mL), H₂O (5 mL), AD-mix-α (1.620 g), and MeSO₂NH₂ (110 mg, 1.156 mmol, 1.0 eq.). The mixture was stirred at rt until both phases were clear, and then cooled to 0°C, whereupon the inorganic salts partially precipitated. Xanthorrhizyl acetate (**6**) (300 mg, 1.152 mmol) was added at once, and the heterogeneous slurry was stirred vigorously at 0 °C for 117 h. The reaction was quenched at 0 °C by addition of Na₂SO₃ (1.73 g) and then warmed to rt and stirred for 35 minutes. The reaction mixture was extracted with ethyl acetate (3 x 10 mL) and the combined organic extract was washed with 2N KOH solution (2 x 20 mL) and dried over anhydrous $MgSO_4$. The ethyl acetate extract was evaporated to gave crude diol which was chromatographed on silica gel (Hex/EtOAc = 4/6) to provide **acetoxydiol (7)** (169.5 mg, 50%) as a colourless oil: R$_f$ = 0.34 (PE/Et₂O, 1/9); $[\alpha]_D$ –38.2° (*c* 1.02, CHCl₃); IR (neat) 3418, 2928, 1747, 1506, 1219 cm^{-1}; ^{1}H NMR (CDCl₃) δ 1.07 (3H, s, H-12), 1.13 (3H, s, H-13), 1.15 (1H, m, H-9'), 1.24 (3H, d, *J* 6.9 Hz, H-15), 1.40 (1H, m, H-9), 1.62 (1H, m, H-8'), 1.83 (1H, m, H-8), 2.02 (1H, d, *J* 5.1 Hz, OH), 2.14 (3H, s, H-14), 2.31 (3H, s, OAc), 2.70 (1H, sext, H-7), 3.32 (1H, ddd, *J* 10.5, 5.1, 2.1 Hz), 6.83 (1H, d, *J* 1.8 Hz, H-2), 6.97 (1H, dd, *J* 7.8, 1.8 Hz, H-6), 7.14 (1H, d, *J* 7.8 Hz, H-5); ^{13}C NMR δ 15.8 (C-14), 20.7 (C-17), 22.6 (C-15), 23.2 (C-12), 26.4 (C-13), 29.5 (C-9), 34.9 (C-8), 39.1 (C-7), 73.1 (C-11), 78.4 (C-10), 120.4 (C-6), 124.7 (C-2), 127.3 (C-4), 131.0 (C-5), 146.2 (C-1), 149.3 (C-3), 169.3 (C-16); EIMS *m/z* 276 [M–H₂O]$^+$ (14), 217 (33), 194 (79), 175 (100), 148 (74), 135 (88).

(10*S*)-10,11-dihydro-10,11-dihydroxy-xan-thorrhizol (2). A solution of acetoxydiol (**7**) (65.6 mg, 0.223 mmol) in 2 mL of methanol and 1 mL of water was treated with saturated solution of sodium bicarbonate (1 mL) and stirred at rt for 17 h. The solution was acidified with 10% HCl solution and extracted with ethyl acetate (3 x 10 mL). The combined organic extract was washed with saturated solution of NaHCO₃ (2 x 10 mL) and dried over anhydrous $MgSO_4$. The solvent was evaporated and the crude product was chromatographed on silica gel (Hex/EtOAc = 3/7 to EtOAc 100%) to give **triol (2)** (44.6

mg, 79.2%) as a colourless oil: $R_f = 0.29$ (PE/Et$_2$O, 1/9); $[\alpha]_D$ −64.7° (*c* 0.51, MeOH); IR (neat) 3361, 2960, 1619, 1260, cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.08 (3H, s, H-12), 1.15 (3H, s, H-13), 1.18 (1H, m, H-9'), 1.23 (3H, d, *J* 6.9 Hz, H-15), 1.40 (1H, m, H-9), 1.60 (1H, m, H-8'), 1.86 (1H, m, H-8), 2.21 (3H, s, H-14), 2.64 (1H, sext, H-7), 3.37 (1H, br d, *J* 10.2 Hz, H-10), 5.06 (1H, s, OH), 6.61 (1H, d, *J* 1.8 Hz, H-2), 6.66 (1H, dd, *J* 7.8, 1.8 Hz, H-6), 7.02 (1H, d, *J* 7.8 Hz, H-5); ^{13}C NMR δ 15.4 (C-14), 23.0 (C-12), 23.2 (C-15), 26.5 (C-13), 29.6 (C-9), 35.0 (C-8), 39.3 (C-7), 73.3 (C-11), 78.6 (C-10), 113.3 (C-2), 119.4 (C-6), 121.3 (C-4), 130.9 (C-5), 146.4 (C-1), 153.9 (C-3); EIMS *m/z* 252 (29) [M$^+$, C$_{15}$H$_{24}$O$_3$], 194 (53), 175 (64), 148 (89), 135 (100).

(7*R*,10*R*)-Acetoxydiol (8). Xanthorrhizyl acetate (**6**) (300 mg, 1.152 mmol) was dihydroxylated in the presence of AD-mix-β (1.62 g) and MeSO$_2$NH$_2$ (110 mg, 1.156 mmol, 1.0 eq.) under the same conditions as described for (7*R*,10*S*)-(**7**) to afford **acetoxydiol (8)** (119.5 mg, 35.2%) as a colourless oil: $R_f = 0.34$ (PE/Et$_2$O, 1/9); $[\alpha]_D$ +5.37° (*c* 1.08, CHCl$_3$); IR (neat) 3416, 2928, 1746, 1215 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.09 (3H, s, H-12), 1.11 (3H, s, H-13), 1.23 (3H, d, *J* 6.9 Hz, H-15), 1.31 (2H, m, H-9 and H-9'), 1.63 (1H, m, H-8'), 1.84 (1H, m, H-8), 2.14 (3H, s, H-14), 2.31 (3H, s, OAc), 2.67 (1H, sext, H-7), 3.21 (1H, br d, *J* 9.6 Hz, H-10), 6.83 (1H, d, *J* 1.8 Hz, H-2), 6.97 (1H, dd, *J* 7.8, 1.8 Hz, H-6), 7.14 (1H, d, *J* 7.8 Hz, H-5); ^{13}C NMR δ 15.8 (C-14), 20.8 (C-17), 22.2 (C-15), 23.1 (C-12), 26.4 (C-13), 29.8 (C-9), 35.2 (C-8), 39.6 (C-7), 73.1 (C-11), 78.5 (C-10), 120.3 (C-6), 124.7 (C-2), 127.3 (C-4), 130.9 (C-5), 146.7 (C-1), 149.3 (C-3), 169.4 (C-16); EIMS *m/z* 276 [M–H$_2$O]$^+$ (4), 194 (72), 175 (100), 148 (66), 135 (88).

(10*R*)-10,11-dihydro-10,11-dihydroxyxan-thorrhizol (3). Acetoxydiol (**8**) (30 mg, 0.102 mmol) was treated the same way as (10*S*)-(**2**) to give **triol (3)** (18.7 mg, 72.8%) as a colourless oil: $R_f = 0.29$ (PE/Et$_2$O, 1/9); $[\alpha]_D$ +3.33° (*c* 0.30, MeOH); IR (neat) 3382, 2962, 1619, 1256 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.11 (3H, s, H-12), 1.14 (3H, s, H-13), 1.22 (3H, d, *J* 6.9 Hz, H-15), 1.34 (2H, m, H-9 and H-9'), 1.58 (1H, m, H-8'), 1.85 (1H, m, H-8), 2.21 (3H, s, H-14), 2.61 (1H, sext, H-7), 3.30 (1H,

dd, *J* 9.9, 2.1 Hz, H-10), 6.62 (1H, d, *J* 1.8 Hz, H-2), 6.67 (1H, dd, *J* 7.8, 1.8 Hz, H-6), 7.02 (1H, d, *J* 7.8 Hz, H-5); ^{13}C NMR δ 15.3 (C-14), 23.1 (C-15), 23.4 (C-12), 26.5 (C-13), 29.9 (C-9), 35.5 (C-8), 39.7 (C-7), 73.1 (C-11), 78.8 (C-10), 113.4 (C-2), 119.1 (C-6), 121.1 (C-4), 130.9 (C-5), 146.9 (C-1), 153.8 (C-3); EIMS *m/z* 252 (11) [M$^+$, C$_{15}$H$_{24}$O$_3$], 234 (2) [M–H$_2$O]$^+$, 148 (47), 135 (100).

Curcuquinone (9). To a stirred solution of Fremy's salt (4.39 g, 16.36 mmol, 2.5 eq.) in NaH$_2$PO$_4$–Na$_2$HPO$_4$ buffer (270 mL, pH 6.90) was added xanthorrhizol (**1**) (1.43g, 6.549 mmol) dissolved in CH$_2$Cl$_2$ (10 mL). The reaction mixture was left to stir under a nitrogen atmosphere for 53 h. The CH$_2$Cl$_2$ layer was separated and the aqueous layer was extracted with CH$_2$Cl$_2$ (8 x 25 mL). The combined organic extract was dried over anhydrous MgSO$_4$, filtered and concentrated to yield the crude quinone as a yellow oil. The crude quinone was chromatographed on silica gel with PE:Et$_2$O = 97/3 as an eluent to give **curcuquinone (9)** (0.8943g, 38.1%) as a yellow oil: $R_f = 0.47$ (PE/Et$_2$O, 9/1); $[\alpha]_D$ − 4.58° (*c* 2.62, CHCl$_3$) {lit. [McEnroe et al., 1978)] $[\alpha]_D$ −1.3° (*c* 9.1, CHCl$_3$)}. IR (neat) 2924, 1735, 1655, 1242 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.08 (3H, d, *J* 6.9 Hz, H-15), 1.34-1.50 (2H, m, H-8), 1.52 (3H, s, H-12), 1.63 (3H, s, H-13), 1.93 (2H, m, H-9), 2.01 (3H, d, *J* 1.8, H-14), 2.83 (1H, sext, H-7), 5.02 (1H, tt, *J* 5.2, 1.5Hz, H-10), 6.48 (1H, d, *J* 0.9 Hz, H-6), 6.56 (1H, q, *J* 1.8 Hz, H-3); EIMS *m/z* 232 (4) [M$^+$, C$_{15}$H$_{20}$O$_2$], 151 (100), 122 (42).

Curcuhydroquinone (10). Na$_2$S$_2$O$_4$ (750 mg, 4.308 mmol) was added to curcuquinone (**9**) (200 mg, 0.861 mmol) in THF/H$_2$O (3:2, 10 mL), and the mixture was stirred at rt for 4 h 30 min. The THF layer was separated and the aqueous layer was extracted with Et$_2$O (3 x 10 mL). The combined organic extract was washed with brine and dried over anhydrous MgSO$_4$. Evaporation of the solvent yielded **curcuhydroquinone (10)** as white solids (175.1 mg, 86.7%): mp 93-96 °C (lit. [Miller et al., 1995] mp 86-87 °C); $R_f = 0.34$ (PE/Et$_2$O, 7/3); $[\alpha]_D$ −48.3° (*c* 0.89, CHCl$_3$) {lit. [Yoshimura et al., 2003] $[\alpha]_D$ −48.0° (c 2.78, CHCl$_3$)}; IR (KBr pellet) 3358, 2926, 1188 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.18 (3H, d, *J* 6.9 Hz, H-15), 1.52 (3H, s, H-12), 1.54-1.64 (2H, m, H-8), 1.66 (3H, s, H-13), 1.91 (2H, m,

H-9), 2.15 (3H, s, H-14), 2.92 (1H, sext, H-7), 5.10 (1H, t with further unresolved couplings, H-10), 6.54 (1H, s, H-3), 6.56 (1H, s, H-6); ^{13}C NMR δ 15.4 (C-14), 17.7 (H-12), 21.1 (C-15), 25.7 (C-13), 26.0 (C-9), 31.4 (C-7), 37.3 (H-8), 113.4 (C-6), 117.9 (C-3), 121.7 (C-4), 123.8 (C-10), 131.8 (C-1), 132.1 (C-11), 146.7 (C-2), 147.8 (C-5); EIMS *m/z* 234 (54) [M$^+$, C$_{15}$H$_{22}$O$_2$], 112 (100).

Curcuhydroquinone diacetate (11). A mixture of curcuhydroquinone (**10**) (325 mg, 1.387 mmol) in CH$_2$Cl$_2$ (5 mL), triethylamine (246 mg, 4.163 mmol, 3.0 eq.), acetic anhydride (425 mg, 4.163 mmol, 3.0 eq.), and DMAP (35 mg, 0.286 mmol, 0.2 eq.) were stirred for 22 h at rt. The reaction mixture was washed with 2N HCl solution (3 x 5 mL) followed by saturated solution of NaHCO$_3$ (2 x 10 mL). The organic layer was dried over anhydrous MgSO$_4$. The solvent was evaporated in vacuo and the crude was chromatographed on silica gel with Hex/Et$_2$O = 9/1 to give **curcuhydroquinone diacetate (11)** (0.3362 mg, 78.6%) as a pale yellow oil: R$_f$ = 0.44 (PE/Et$_2$O, 7/3); [α]$_D$ –28.2° (*c* 1.00, CHCl$_3$); IR (neat) 2928, 1761, 1504, 1205, cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.16 (3H, s, d, *J* 7.2 Hz, H-15), 1.46-1.62 (2H, m, H-8), 1.53 (3H, s, H-12), 1.66 (3H, s, H-13), 1.90 (2H, m, H-9), 2.12 (3H, s, H-14), 2.29 (3H, s, OAc), 2.30 (3H, s, OAc), 2.79 (1H, sext, *J* 5.4 Hz, H-7), 5.06 (1H, t, *J* 5.4 Hz), 6.84 (1H, s, H-6), 6.87 (1H, s, H-3); ^{13}C NMR (75 MHz, CDCl$_3$) δ 15.9 (C-14), 17.6 (C-12), 20.8 (CO*Me*), 20.9 (CO*Me*), 20.9 (C-15), 25.7 (C-13), 25.9 (C-9), 32.0 (C-7), 38.9 (C-8), 120.5 (C-6), 124.2 (C-10), 124.5 (C-3) 128.4 (C-4), 131.0 (C-11), 137.9 (C-1), 145.8 (C-2), 147.3 (C-5), 168.9 (C=O), 169.3 (C=O); EIMS *m/z* 318 (17) [M$^+$, C$_{19}$H$_{26}$O$_4$], 234 (100), 164 (21), 151 (82).

Helibisabonol A diacetate (4). Curcuhydroquinone diacetate (**11**) (170 mg, 0.551 mmol) was dihydroxylated in the presence of AD-mix-α (0.772 g) and MeSO$_2$NH$_2$ (53 mg, 0.357 mmol) under the same conditions as described for (7*R*,10*S*)-(**7**) to afford crude **4** as a sticky yellow oil. The residue was purified by column chromatography (silica gel, Hex/EtOAc = 4/6) to afford **helibisabonol A diacetate (4)** (120 mg, 61.8 %) as white solids: mp 78-81 °C; R$_f$ = 0.40 (Et$_2$O/EtOAc, 4/1); [α]$_D$ –37.5° (*c* 0.24,

CHCl$_3$); IR (KBr pellet) 3343, 2975, 1762, 1212 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.08 (3H, s, H-12), 1.14 (3H, s, H-13), 1.36-1.93 (4H, m, H-8 and H-9), 2.15 (3H, s, H-14), 2.32 (2 x 3H, s, H-17 and H-19), 2.86 (1H, sext, H-7), 3.29 (1H, br d, *J* 10.5 Hz, H-10), 6.88 (1H, s, H-3), 6.93 (1H, s, H-6); ^{13}C NMR δ 15.8 (C-14), 20.8 (C-17), 20.9 (C-15), 21.7 (C-19), 23.3 (C-13), 26.2 (C-12), 29.4 (C-9), 32.8 (C-7), 33.8 (C-8), 73.0 (C-11), 78.4 (C-10), 124.6 (C-3 and C-6), 128.6 (C-4), 137.1 (C-1), 145.8 (C-2), 147.2 (C-5), 169.8 (C-16), 170.3 (C-18); EIMS *m/z* 310 (1) [M–CH$_2$CO]$^+$, 250 (46), 151 (100), 59 (34).

Bioassays

Brine shrimp lethalities for xanthorrhizol (**1**) was determined as described in the literature (Meyer et al., 1982).

Antimicrobial assay was carried out against six types of bacteria, *S. aureus*, *B. subtilis*, *B. cereus*, *P. aeruginosa*, *S. faecalis*, *S. typhii* and against three types of fungi, *Trichoderma sp.*, *C. cladospoides* and *C. albicans* by agar diffusion method (Hewitt et al., 1989). Minimum inhibitory concentrations (MIC) were determined by the standard broth microdilution method.

Results and discussion

Hydrodistillation of the chopped fresh rhizomes of *C. xanthorrhiza* yielded essential oil in 0.91 % yield and xanthorrhizol was isolated from the essential oil by vacuum liquid chromatography in 38.7% yield based on the crude essential oil.

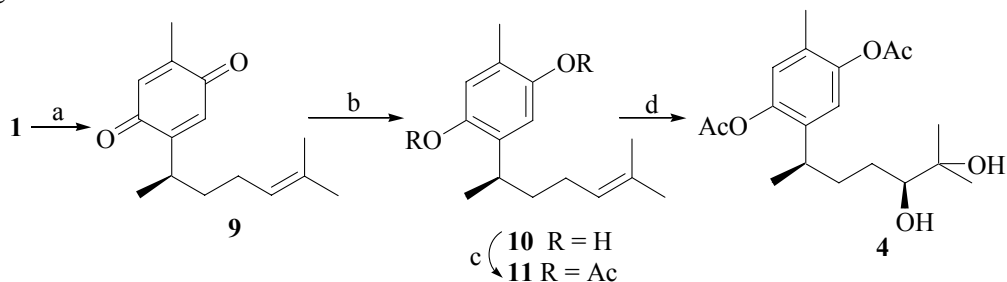The synthetic route to triols **2** and **3** are outlined in Scheme 1.

First, xanthorrhizol (**1**) was treated with Ac$_2$O/py to furnish xanthorrhizyl acetate (**6**). Acetate (**6**) was subjected to AD employing AD-mix-α (Kolb et al., 1994) to give the diol **7** in 50% yield. The diastereomeric excess was >98% as determined by ^{1}H NMR analysis of its (*S*)-MTPA [α-methoxy-α-(trifluoromethyl)-phenylacetic acid] ester derivative. The absolute configuration of the newly formed stereogenic centre was deduced to be *S* by the modified Mosher method (Ohtani et al., 1991). The diol **7** was treated with aqueous sodium bicarbonate to give triol **2**. The overall yield of **2** was 28.5%. The diastereomer of **2**, (7*R*,10*R*)-**3** was obtained in 18.5% overall yield, following the same

sequence of reactions except AD-mix-β was used instead.
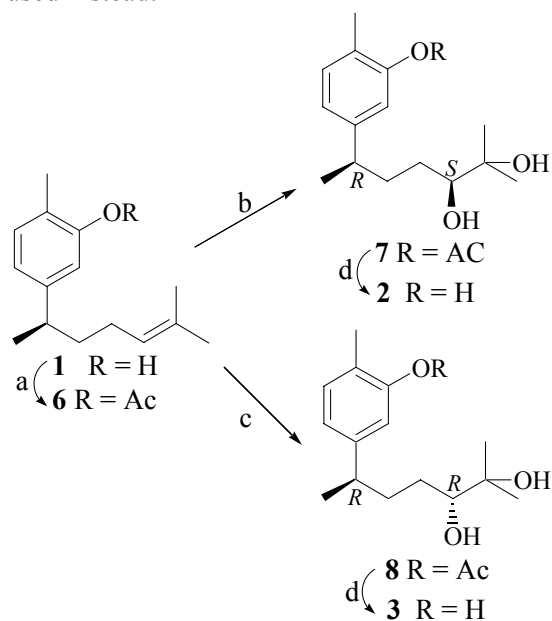
SCHEME 1 *Reagents and conditions*: (a) Ac$_2$O, py, rt, 72%; (b) AD-mix-α, MeSO$_2$NH$_2$, *t*-BuOH:H$_2$O (1:1), 0 °C, 50%; (c) AD-mix-β, MeSO$_2$NH$_2$, *t*-BuOH:H$_2$O (1:1), 0 °C, 35.2%; (d) sat. NaHCO$_3$, MeOH:H$_2$O (2:1), rt, 79.2% (**2**), 72.8% (**3**).

The synthetic route to helibisabonol A diacetate (**4**) is summarised in Scheme 2. Xanthorrhizol (**1**) was oxidised with potassium nitrosodisulphonate (Fremy's salt) to give curcuquinone (**9**), which was subsequently reduced to curcuhydroquinone (**10**) by sodium dithionite. The spectroscopic properties of **9** and **10** were in good agreement with the literature data. Curcuhydroquinone (**10**) was acetylated to give curcuquinone diacetate (**11**). This was converted to helibisabonol A diacetate (**4**) by Sharpless AD using AD-mix-α. The diastereomeric excess was >98% [(*S*)-MTPA ester] and the absolute configuration at C-10 in **4** was determined to

be *S* based on modified Mosher method (Ohtani et al., 1991).

Bioassays

Xanthorrhizol (**1**) displayed toxicity towards *Artemia salina* (LC$_{50}$ = 25.91 ppm) and cytotoxicity against HL-60 leukimia cell line (IC$_{50}$ = 0.4 μg/mL).

Xanthorrhizol (**1**) was submitted for antibacterial and antifungal assays. Xanthorrhizol (**1**) showed antibacterial activity against *S. aureus*, *B. subtilis*, *B. cereus*, *P. aeruginosa*, *S. faecalis* and *S. typhii* With MIC values in the range of 3.125-50 μg/mL. The results showed that **1** was most active in inhibiting the *S. faecalis* with MIC value of 3.125 μg/mL. Xanthorrhizol (**1**) also displayed potent antifungal activity against *Trichoderma sp.*, *C. cladosporoides* and *C. albicans* at MIC values in the range of 12.5-50 μg/mL (Table 1).

TABLE 1 Antimicrobial activity of xanthorrhizol (**1**).

Organism	MIC (μg/mL)
S. aureus	25
B. substilis	50
B. cereus	25
P. aeruginosa	25
S. faecalis	3.125
S. typhii	50
Trichoderma sp.	12.5
C. cladosporoides	12.5
C. albicans	50

Methyl ether of xanthorrhizol showed high PAF activity at IC$_{50}$ = 40.9 μM, however xanthorrhizol (**1**) and xanthorrhizyl acetate (**6**) were not active in PAF activity.

SCHEME 2 *Reagents and conditions*: (a) (KO$_3$S)$_2$NO• (Fremy's salt), CH$_2$Cl$_2$, NaH$_2$PO$_4$-Na$_2$HPO$_4$, pH 6.90, rt, 38.1%; (b) Na$_2$S$_2$O$_4$, THF-H$_2$O (3:2), rt, 87%; (c) DMAP, Et$_3$N, Ac$_2$O, rt, 88%; (d) AD-mix-α, MeSO$_2$NH$_2$, *t*-BuOH:H$_2$O (1:1), 0 °C, 62%.

Compounds **2**, **7-8** and **10-11** were subjected to anti-inflammatory test using human whole blood prostaglandin E2 inhibition assay. Preliminary screening would suggested that curcuhydroquinone (**10**) and curcuhydroquinone diacetate (**11**) are worth further investigation with **11** showed the most potent anti-inflammatory activity.

Besides that, xanthorrhizol (**1**) and its simple derivatives showed tyrosinase inhibition activity against L-DOPA.

The results in this study showed that xanthorrhizol is a potential precursor for bisabolane-type sesquiterpenoids, which has been shown by the first enantioselective synthesis of triols **2** and **3**, a facile and rapid synthesis of (–)-curcuquinone (**9**) and (–)-curcuhydroquinone (**10**) as well as synthesis of helibisabonol A diacetate (**4**). Xanthorrhizol and its derivatives showed interesting bioactivities.

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