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New triterpenes from the bark of *Canarium asperum*

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ABSTRACT

*Chemical investigation of the dichloromethane extract of the resin from the bark of Canarium asperum led to the isolation of a mixture of new triterpene diastereomers, asperol a (**1a**) and asperol b (**1b**) in a 3:2 ratio. The structures of **1a** and **1b** were elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by mass spectrometry. β-amyrin and α-amyrin were also obtained as the major constituents of the resin.*

Keywords: *Canarium asperum*, Burseraceae, asperol a, asperol b, β-amyrin, α-amyrin

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INTRODUCTION

Canarium asperum subsp. *asperum* var. *asperum* Leenh. (Burseraceae) constitutes a highly polymorphous variety of the species that is endemic in the Malesian region, where it grows apparently well both in the primary and secondary forests or even in savannahs but mainly at low altitudes [1]. It is a native of Brunei Darussalam, Indonesia (Jawa), Malaysia, Papua New Guinea, Philippines and Solomon Islands [2]. Although the wood furnishes little durability for construction works, the resin from this tree (known in the Philippines as *pagsahingin* or *sahing*) is a popular source of fuel and light [1, 3-4]. As early as 1920, West and Brown found that the principal constituent of the volatile oil of *pagsahingin* resin is d-pinene and suggested that this species along with the world renowned Manila elemi (*Canarium luzonicum*) produce terpenes which vary from tree to tree. To date, there is no other reported study on the chemical constituents of *C. asperum*. However, the *C. asperum* congener, *C. ovatum* which is locally known as pili has been investigated chemically. A recent study reported the isolation of sesquiterpene alcohols, triterpene alcohols and triterpene acids from the resin of *C. ovatum.* The three sesquiterpene alcohols, cryptomeridiol, 4-epicryptomeridiol and cadin-1(14)-ene-7a,11-diol from *C. ovatum* resin were found to be potential skin whitening agents [5]. Previous studies reported that the resins from various *Canarium* species contain α-amyrin and β-amyrin as the major constituents [6 –9] and tirucallane-type triterpene acids as minor constituents [6, 10].

We report herein the isolation and structure elucidation of new triterpene diastereomers, asperol a (**1a**) and asperol b (**1b**) (Figure 1), together with the major triterpene constituents, β-amyrin and α-amyrin from the dichloromethane extract of *C. asperum* resin. To the best of our knowledge, this is the first report on the isolation of these compounds from *C. asperum.*

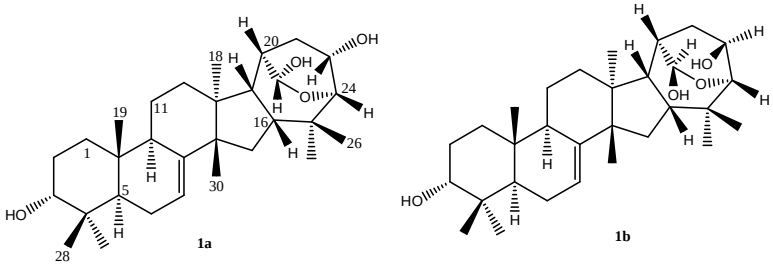

Fig. 1. Asperol a (1a) and asperol b (1b) from *Canarium asperum*

MATERIALS AND METHODS

General Experimental Procedures

NMR spectra were recorded on a Varian VNMRS spectrometer in $CDCl_3$ at 600 MHz for ^1H NMR and 150 MHz for ^{13}C NMR spectra. HR-ESI-MS was obtained using a Shimadzu LCMS-IT-TOF mass spectrometer. UV spectrum was measured on a Hitachi U-3310 spectrophotometer. IR spectrum was recorded on a Nicolet Avatar 320 FT-IR spectrometer. Column chromatography was performed with silica gel 60 (70-230 mesh). Thin layer chromatography was performed with plastic backed plates coated with silica gel F_{254} and the plates were visualized by spraying with vanillin/H_2SO_4 followed by warming.

Sample Collection

The resin from the bark of *Canarium asperum* subsp. *asperum* var. *Asperum* Leenh. (Fam. Burseraceae) was collected from Barangay San Vicente, Roxas Oriental Mindoro, Philippines in May 2012 with gratuitous permit issued by the Sadikhabanan Buhid Inc. The sample was authenticated by one of the authors (EHM). A voucher specimen #DDR001 was deposited at the Biology Department, De La Salle University, Manila, Philippines.

Isolation

The resin of *C. asperum* (55 g) was soaked in CH_2Cl_2 for three days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (42 g). A glass column 20 inches in height and 2.0 inches internal diameter was packed with silica gel. The crude CH_2Cl_2 extract from the leaves were fractionated by silica gel chromatography using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. One hundred milliliter fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same *Rf* values were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained. A glass column 18 inches in height and 1.0 inch internal diameter was used for the fractionation of the crude extracts. Five milliliter fractions were collected. Fractions with spots of the same *Rf* values were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained. A glass column 12 inches in height and 0.5 inch internal diameter was used for the rechromatography. Two milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

The 20% and 30% acetone in CH_2Cl_2 fractions from the chromatography of the crude extract were combined and rechromatographed (4×) using10% EtOAc in petroleum ether to afford a mixture of β-amyrin and α-amyrin (2 g) after washing with petroleum ether. The 60% to 70% acetone in CH_2Cl_2 fractions from the chromatography of the crude extract were combined and rechromatographed (6×) using CH_3CN:Et_2O:CH_2Cl_2 (1:1:8 by volume ratio) to afford a mixture of **1a** and **1b** (15 mg) after trituration with petroleum ether.

Asperol a (**1a**): colorless solid. UV (MeOH) λ_{max} (logε) 202 (4.70), 270 (3.92); IR (KBr) ν_{max} (cm^{-1}) 3419 (O-H), 2943, 2878, 1639, 1458, 1378, 1116, 1066, 972.^1H NMR (600 MHz, $CDCl_3$):δ1.34, 1.45 (H_2-1), 1.58, 1.92 (H_2-2), 3.45 (H-3, brs), 1.77 (H-5), 1.90, 2.04 (H_2-6), 5.24 (H-7, brs), 2.35 (H-9), 1.48, 1.58 (H_2-11), 1.34, 1.45 (H_2-12), 1.48, 1.58 (H_2-15), 1.78 (H-16), 2.04 (H-17), 0.83 (H_3-18, s), 0.75 (H_3-19, s), 2.00 (H-20), 5.30 (H-21, brs), 1.72, 2.01 (H_2-22), 3.85 (H-23, m), 2.82 (H-24, d, J = 7.8 Hz),1.28 (H_3-26, s), 1.29 (H_3-27, s), 0.89 (H_3-28, s), 0.91 (H_3-29, s), 0.99 (H_3-30, s), 2.76 (21-OH, brs);^{13}C NMR (150 MHz, $CDCl_3$): δ 31.18 (C-1), 25.35 (C-2), 76.29 (C-3), 37.39 (C-4), 44.61 (C-5), 23.93 (C-6), 118.15 (C-7), 145.53 (C-8), 48.85 (C-9), 34.80 (C-10), 17.40 (C-11), 31.50 (C-12), 43.54 (C-13), 51.07 (C-14), 34.80 (C-15), 50.39 (C-16), 45.23 (C-17), 23.21 (C-18), 12.93 (C-19), 47.10 (C-20), 97.82 (C-21), 31.57 (C-22), 78.45 (C-23), 67.80 (C-24), 57.25 (C-25), 19.20 (C-26), 25.00 (C-27), 21.77 (C-28), 27.75 (C-9), 27.30 (C-30); HR-ESI-MS *m/z* 472.3553 $[M]^+$($C_{30}H_{48}O_4$).

Asperol b (**1b**): colorless solid. ^1H NMR (600 MHz, $CDCl_3$):δ1.34, 1.45 (H_2-1), 1.58, 1.92 (H_2-2), 3.45 (H-3, brs), 1.77 (H-5), 1.90, 2.04 (H_2-6), 5.24 (H-7, brs), 2.35 (H-9), 1.48, 1.58 (H_2-11), 1.34, 1.45 (H_2-12), 1.48, 1.58 (H_2-15), 1.78 (H-16), 2.04 (H-17), 0.88 (H_3-18, s), 0.75 (H_3-19, s), 2.22 (H-20), 5.36 (H-21, brs), 1.36, 2.12 (H_2-22), 3.94 (H-

23, m), 2.68 (H-24, d, *J* = 7.2 Hz), 1.28 (H_3-26, s), 1.31 (H_3-27, s), 0.89 (H_3-28, s), 0.91 (H_3-29, s), 0.98 (H_3-30, s), 2.66 (21-OH, brs);^{13}C NMR (150 MHz, $CDCl_3$): δ 31.16 (C-1), 25.34 (C-2), 76.25 (C-3), 37.39 (C-4), 44.56 (C-5), 23.91 (C-6), 118.26 (C-7), 145.86 (C-8), 48.44 (C-9), 34.84 (C-10), 17.40 (C-11), 31.46 (C-12), 43.72 (C-13), 50.82 (C-14), 34.82 (C-15), 50.39 (C-16), 45.23 (C-17), 22.57 (C-18), 12.92 (C-19), 49.61 (C-20), 101.81 (C-21), 35.15 (C-22), 76.79 (C-23), 65.35 (C-24), 57.98 (C-25), 19.42 (C-26), 24.93 (C-27), 21.76 (C-28), 27.75 (C-9), 27.11 (C-30); HR-ESI-MS *m/z* 472.3553 $[M]^+$ ($C_{30}H_{48}O_4$).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the resin from the bark of *C. asperum* afforded a mixture of new triterpene diastereomers (**1a** and **1b**) and another mixture of β-amyrin and α-amyrin. The structures of **1a** and **1b** were elucidated by extensive 1D and 2D NMR spectroscopy as follows.

The ^{1}H NMR spectrum of **1** indicated a diastereomeric mixture as deduced from the doubling of some of the ^{1}H NMR resonances in the spectrum (see experimental part). A 3:2 ratio of **1a** and **1b** was deduced from the ^{1}H NMR integrations and intensities of corresponding ^{13}C NMR resonances. The mixture was confirmed by the ^{13}C NMR spectrum (see experimental part) which also showed mostly carbon doublets which increased in chemical shift differences for carbons close to and in the two diastereomeric centers. The structure elucidation of the major compound (**1a**) is discussed, while the minor compound (**1b**) is compared to **1a**.

The ^{1}H NMR spectrum of the major compound (**1a**) (see experimental part) indicated resonances for an olefinic proton at δ 5.24 (brs); oxymethine protons of alcohols at δ 3.45 (brs) and 3.85 (m) and an ether at δ 2.82 (d, *J* = 7.8 Hz); an acetal proton at δ 5.30 (brs); seven methyl singlets at δ 0.75, 0.83, 0.89, 0.91, 0.99, 1.28 and 1.29; and a hydroxyl at δ 2.76 (br s).

The ^{13}C NMR and DEPT spectra of **1a** (see experimental part) gave resonances for thirty carbons as follows: olefinic carbons at δ 145.53 and 118.15; three oxymethine carbons at δ 78.45, 76.29 and 67.80; a hemiacetal carbon at δ 97.82; seven methyl carbons at δ 27.75 and 27.30, 25.00, 23.21, 21.77, 19.20 and 12.93; seven methylene carbons at δ 34.80, 31.57, 31.50, 31.18, 25.35, 23.93 and 17.40; five methine carbons at δ 50.39, 48.85, 47.10, 45.23 and 44.61; and five quaternary carbons at δ 57.25, 51.07, 43.54, 37.39 and 34.80. These resonances suggest a triterpene with a hemiacetal, an ether, an olefin and two alcohol functionalities.

The HR-ESI-MS of **1** gave an *m/z* 472.3553 $[M]^+$ corresponding to a molecular formula of $C_{30}H_{48}O_4$. This indicated an index of hydrogen deficiency of seven. With a double bond deduced from the ^{13}C NMR spectrum, **1** is a mixture of hexacyclic triterpenes.

The COSY spectrum of **1a** indicated four isolated spin systems as follows: H_2-1/H_2-2/H-3; H-5/H_2-6/H-7; H-9/H_2-11/H_2-12; H_2-15/H-16/H-17/H-20/H-21,H_2-22/H-23/H-24 (Fig. 2).

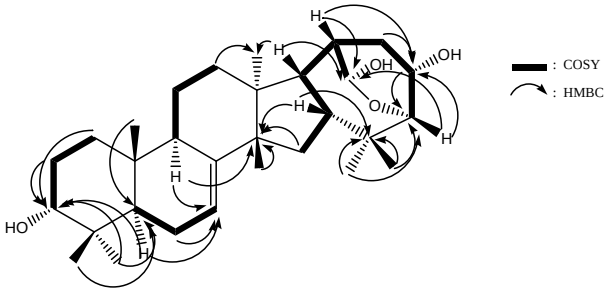

Fig. 2. ^{1}H-^{1}H COSY and Key HMBC correlations of 1a.

Protons attached to carbons were assigned (see experimental part) from HSQC 2D NMR data and the structure of **1a** was elucidated by analysis of the HMBC 2D NMR data: key HMBC correlations are shown in Fig. 2. Thus, one of the oxymethine carbons was assigned to C-3 on the basis of long-range correlations between H_3-28, H_3-29, H_2-1, H_2-2, H-5 and this carbon. The second oxymethine carbon was attributed to C-23 since correlations were observed between H-24, H_2-22 and H-20, and this carbon. The oxymethine carbon of the ether was assigned to C-24 based on correlations between H_3-27, H_3-26, H-23 and H_2-22, and this carbon. The hemiacetal was assigned to C-21 on the basis of long-range correlations between H-24, H_2-22, H-20 and H-17, and this carbon. The methyl singlets (H_3-29, H_3-28 and H_3-19) were assigned based on long-range correlations to C-5. The fourth methyl singlet was attached to C-14 due to long-range correlations between H-9, H_2-15, H-16 and H_3-30, and this carbon. Another methyl singlet

was attributed to C-18 based on correlations between H-17, H_2-12 and this carbon. The two remaining methyl singlets were attached to C-25 since correlations were observed between H_3-27, H_3-26, H-24, H-23, H-17, H-16 and H_2-15, and this carbon. The olefin was assigned to C-7 on the basis of long-range correlations between H-9, H_2-6 and H-5, and this carbon. All long-range correlations are consistent with the structure of **1a**.

The relative stereochemistry of **1a** was deduced from NOESY (Fig. 3). Thus, H-3 was in the equatorial position since it was close in space to H_2-2, H_3-28 and H_3-29. The methyl (H_3-28) was close to another methyl (H_3-19), indicating that they are on the same face of **1a**. No correlation was observed between H-5 and H_3-19, suggesting that they are *trans* to each other. The methine proton (H-5) was close to H-9 which was in turn close to H_3-18, which was also close to H-20 and the hemiacetal proton (H-21), which was in turn close to H-15. No correlation was observed between H_3-18 and H_3-30, indicating that they are *trans* to each other. Correlations were observed between H_3-30, H-16 and H-17, suggesting that they are on the same face of **1a.** Thus, H-16 and H-17 are *cis* to each other. Furthermore, H-17 correlated with H-23 which in turn correlated to H_3-26, but did not correlate with H-24, indicating that H-23 and H-24 are in the opposite faces of **1a**. Finally, H-24 was close to the two methyl protons (H_3-26 and H_3-27). Thus, the relative stereochemistry of **1a** is as shown in Fig. 2. The structure of **1a** was confirmed by the ESIMS of **1** which gave an *m/z* 495.60 $[M+Na]^+$. The trivial name asperol a is suggested for **1a**.

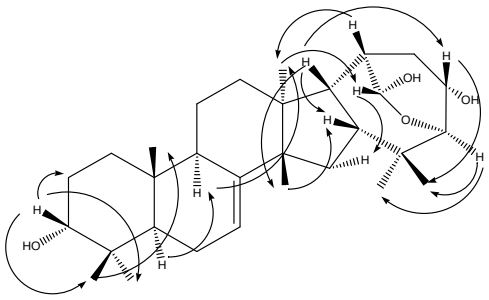

Fig. 3. NOESY correlations of 1a.

The ^{1}H NMR of **1b** (see experimental part) indicated similar resonances to **1a**. The differences lie in the region where their structures differ. Marked differences in the chemical shifts in the ^{1}H NMR of **1b** were observed in the deshielding of H-23 from δ3.85 in **1a**to 3.94in **1b**; the deshielding and shielding of H_2-22 from δ 1.72 and 2.01 to 2.12 and 1.36, respectively; and the shielding of H-24 from δ2.86 to 2.68. These resulted from the inversionof configuration at C-23. This was supported by the NOESY correlations between H-23 and H-24 and H_2-22. The inversion of configuration of the hemiacetal carbon at C-21was deduced from the change in the NOESY correlations of H-21 in **1b** and the deshielding of the hemiacetal proton from δ 5.30 in **1a** to δ 5.36 in **1b**. No correlation was observed between H-21 and H_3-18 and H-15in **1b** which were previously observed in **1a**. In its new position, the hydroxyl attached to C-21 is now close to H_3-18 resulting in the deshielding of these methyl protons from δ 0.83 in **1a** to δ 0.88 in **1b**. This change also resulted in the deshielding of H-20 from δ 2.00 in **1a** to δ 2.22 in **1b**. On the other hand, the hemiacetal hydroxyl was shielded from δ 2.76 in **1a** to δ 2.66 in **1b**.

These changes in the relative stereochemistries at C-21 and C-23 were supported by the shielding and deshielding of the carbons in the hemiacetal ring (C-20 to C-24). The ^{13}C NMR spectrum of **1b** (see experimental part) indicated the shielding of the carbons at C-23 and C-24 from δ 78.45 and 67.80 in **1a** to δ 76.79 and 65.35 in **1b**, respectively. On the other hand, the carbons at C-20, C-21 and C-22 were deshielded from δ 47.10, 97.82 and 31.57 in **1a** to δ 49.61, 101.81 and 35.15 in **1b**, respectively.

The structure of **1b** was further supported by correlations from its 2D NMR (COSY, HSQC and HMBC) data. The HSQC confirmed the ^{1}H and ^{13}C attachments in the hemiacetal ring (C-20 to C-24) in **1b**, while connectivities were verified by COSY and HMBC. Thus, from the COSY spectrum, H-23 is coupled to H-24 and H_2-22, which were in turn coupled to H-20, which was also coupled to H-21, which was finally coupled to the hemiacetal OH. The HMBC spectrum indicated long-range correlations between H-23, H_3-26, H_3-27 and C-24; H-21, H-22, 21-OHand C-20; and H-22 and C-23 which are consistent with the structure of **1b**. The trivial name asperol b is suggested for **1b**.

Asperol a and asperol b resulted from ring closure between C-25 and C-16 in tirucallane-type triterpenes which were earlier reported as minor constituents of various *Canarium* species [6, 10].

The major compounds obtained from the resin are a mixture of β-amyrin and α-amyrinin a 2:3 ratio as deduced from the ^{1}H NMR integrations and intensities of the olefinic^{13}C NMR resonances. The compounds were identified by

comparison of their ^{13}C NMR data with those reported in the literature for β-amyrin and α-amyrin [11]. These triterpenes were previously reported as major constituents of *Canarium* species [6-9].

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