Aspidospermatan–aspidospermatan and eburnane-sarpagine bisindole alkaloids from Leuconotis

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Abstract

Leucofoline and leuconoline, representing the first members of the aspidospermatan–aspidospermatan and eburnane-sarpagine subclasses of the bisindole alkaloids, respectively, were isolated from the Malayan Leuconotis griffithii. The structures of these bisindole alkaloids were established using NMR and MS analysis, and in the case of leuconoline, confirmed by X-ray diffraction analysis. Both alkaloids showed weak cytotoxicity towards human KB cells.

1. Introduction

Plants of the genus Leuconotis (Apocynaceae) are usually woody climbers and occur in Indonesia and Peninsular Malaysia (Ridley, 1923; Markgraf, 1971; Whitmore, 1972). The genus comprises a small group of 10 species, of which three (L. griffithii Hook.f., L. maingayi Dyer ex Hook.f., and L. eugenifolius A.DC.) are found in Peninsular Malaysia. Previous studies of the Malayan L. griffithii and L. eugenifolius have provided in addition to the ring-opened alkaloids, leuconolam and rhazinilam and their derivatives, various strychnan, kopsan, and eburnane derivatives (Goh et al., 1984, 1989), while several yohimbines and the pentacyclic diazaspiro alkaloid, leuconoxine, were reported from L. eugenifolius occurring in Indonesia (Abe and Yamauchi, 1994). The alkaloidal composition of these plants bear a striking similarity to plants of the genus Kopsia (Feng et al., 1983; Zhu et al., 1986; Kam et al., 1993, 1996, 1998b, 1999a,b, 2004b; Varea et al., 1993; Kan et al., 1995; Uzir et al., 1997; Kam and Sim, 1998; Kam and Choo, 2004; Zhou et al., 2006; Lim et al., 2007a,b; Lim and Kam, 2007; Subramaniam et al., 2007, 2008a,b; Kam and Lim, 2008; Subramaniam and Kam, 2008; Low et al., 2009). More recent studies of L. griffithii and the previously uninvestigated L. maingayi, have resulted in the discovery of new indole and bisindole alkaloids (Gan et al., 2009a,b; Gan and Kam, 2009; Hirasawa et al., 2010). We now report the isolation, structure, and biological activity of two new bisindole alkaloids, representing first members of the aspidospermatan–aspidospermatan and eburnane-sarpagine type bisindole alkaloids (1, 2) from the stem-bark extract of L. griffithii.

2. Results and discussion

Leucofoline (1) was isolated as a light yellowish oil, [α]D +113 (CHCl3, c 0.28). The UV spectrum showed absorption maxima at 214 and 273 nm indicating the presence of an indolenine chromophore. The ESIMS of 1 showed a quasi molecular ion at m/z 557, and HRESIMS measurements established the molecular formula as C38H44N4 (DBE or double bond equivalents 19). The 13C NMR spectrum (Table 1) accounted for all of 38 carbon resonances, comprising two methyl, twelve methylene, fourteen methine, and ten quaternary carbons. The 1H NMR spectrum showed absorption resonances for two aromatic moieties, comprising two methyl, twelve methylene, fourteen methine, and ten quaternary carbons. The 13C NMR spectrum showed the presence of eight aromatic hydrogens corresponding to two substituted aromatic moieties, and two isolated methylenes (δH 1.86, 3.30; δC 42.6; δH 3.75, 4.11; δC 48.4). In addition to the two aromatic CHCHCHCH units, the main partial structures revealed by the COSY
and HSQC data were two NCHCHCH2CH3 fragments, which are characteristic of the N-(4)-(C(21)-(C(20)-(C(19)-(C(18)) fragment of the aspidospermatan- or condylocarpine-type alkaloids.

Examination of the 1H and 13C NMR data showed that one unit of the bisindole corresponds to the known alkaloid, epi-condylocarpine (3) (Schumann and Schmid, 1963), except for C-16 which is a quaternary carbon in 1 instead of a methylene in 3. This provided the first indication that branching of the bisindole from this indolene unit is from C-16, since there was no evidence of substitution at the other carbons of the condylocarpine-like unit.

Examination of the remaining fragments which correspond to those constituting the other condylocarpine unit, revealed a similar ring system as 3, except for the presence of an enamine double bond instead of an indolene. This left two isolated methylene groups, one each attached to the indolic N-1 and to C-16' of the second indole unit.

This is consistent with the observed shifts of these methylenes (δ 3.75, 4.11, NH; δ 1.86, 3.30, CO2Me) as well as the HMBC data (Fig. 1). Both of these methylenes are linked to C-16 of the first or condylopyridine-like unit to forge a tetrahydropyridine ring incorporating C-16 as a spirocyclic center. This was supported by the observed 1J and 2J correlations from H-22 to C-16, and from H-22' to C-2, respectively, as seen in the HMBC spectrum. This mode of branching in leucofoline (1) is somewhat similar to that in the Aspidosperma-Aspidosperma bisindole, anhydrohazuntiphyllidine (Bui et al., 1991), and is also in agreement with the HMBC data (Fig. 1).

The NOE data showed that the relative configurations of the monomeric units are similar to those of condylocarpine. The configuration at the spirocyclic center, C-16, was determined to be R,
from the observed H-20/H-22 a reciprocal NOEs, which are possible only if C-16 is R (Fig. 2a). In the converse case (C-16S), these two hydrogens will be directed away from each other and would therefore not be expected to show any NOE (Fig. 2b).

Leucofloline (1) represents the first example of a bisindole of the aspidospermatan–aspidospermatan type. A possible pathway to 1 is shown in Scheme 1 involving conjugate addition via the indolic nitrogen of the condylolcarpine-type precursor 5 (or 16-methylenecondyloline) (Walser and Djerassi, 1965) onto the imine to effect the N-1 to C-16 link, followed by a subsequent conjugate addition of an enamine to forge the spirocyclic ring system.

Leuconoline (2) was isolated as pale yellowish crystals from EtOH, [α]D +142 (CHCl3, c 0.49). The IR spectrum indicated the presence of OH (3401 cm−1) and ester carbonyl (1713 cm−1) functions. The UV spectrum showed absorption maxima at 230 and 285 nm, characteristic of an indole chromophore. The EIMS showed a molecular ion at m/z 646, and HREIMS measurements established the molecular formula as C40H46N4O4. The 1H NMR spectrum of 2 (Table 1) gave a total of 40 carbon resonances (three methyl, eleven methylene, twelve methine and fourteen quaternary carbons) in agreement with the molecular formula from HREIMS. The 1H NMR spectrum showed the presence of six aromatic hydrogens, four of which correspond to the four contiguous hydrogens of an indole unit as indicated from the COSY spectrum. The remaining two aromatic hydrogens were seen as a pair of AB doublets at δ 6.62 and 7.05. The 13C NMR spectrum also showed the presence of a broad one-H singlet at δ 7.98 (exchanged with D2O), a methoxy signal at δ 2.97 which is associated with a methyl ester moiety, signals due to an ethyl side chain at δ 0.97; 1.54, 2.27; another pair of signals due to an ethylidene side chain at δ 1.64 and 5.38; an oxymethylene associated with a hydroxymethyl group at δ 3.59 and 3.80; a singlet at δ 4.05 due to an isolated methine (H-21'); and an isolated methylene signal at δ 3.52 and 3.61 (H-21). The observation of ester carbonyl and oxymethylene resonances at δ 175.2 and δ 69.4, respectively, are consistent with the presence of the methyl ester and hydroxymethyl groups. The broad singlet at δ 7.98 is attributed to the indolic NH associated with the disubstituted indole moiety. This is based on the observed NOE between NH and H-12 (δ 7.05) of the disubstituted indole unit which also allowed assignment of the aromatic AB doublets to H-11 and H-12. Since the molecular formula showed the presence of four oxygen atoms and three have already been accounted for, the remaining oxygen must be due to a phenolic OH associated with the disubstituted indole moiety. This was further supported by acetylation (Ac2O/DMAP), which yielded a diacetate derivative 4 (δH 1.72 and 1.98; δC 20.2, 169.0, 209.3, 170.8; see Section 3).

Examination of the NMR chemical shifts as well as the 2-D COSY and HETCOR data revealed that one unit of the bisindole corresponds to an eburnane moiety. This is supported by examination of the 13C NMR data which showed a close correspondence to those of eburnamine (Kam et al., 1992) or bisindole alkaloids incorporating an eburnane half, such as the recently found bisindole, leucophyllidine (7) (Kam and Choo, 2006; Gan et al., 2009b). The H-16 signal was observed as a doublet of doublets (J = 12, 4.4 Hz) indicating branching of the bisindole from C-16 of the eburnane unit (Kam and Choo, 2006; Kam and Lim, 2008). This conclusion receives additional support from the observation of long-range correlation from the eburnane H-16 to the aromatic C-10 of the other indole unit (vide infra). The configurations at C-20 and C-21 of the eburnane unit were deduced to be 20R and 21R (20j, 21β) on the basis of a presumed common biogenetic origin, since the other eburnane alkaloids found in the plant (Gan et al., 2009b) such as (−)-eburnamine, (+)-isoeburnamine, (+)-eburnamonine, and (+)-eburnamenine, all belong to the 20R, 21R enantiomeric group (Kam et al., 1993, 1999b; Kam and Choo, 2006; Kam and Lim, 2008).

The other unit constituting the bisindole, after discounting the eburnane half, incorporates a dissubstituted indole moiety, an indolic NH, a methyl ester, a hydroxymethyl, an aminomethylene, an ethylidene side chain, as well as NCHCH2 and NCHCH2CH partial bonds. The unusual shielding of the ester methyl at δ 3.59 and 3.80 due to an isolated methine (H-21′); and an isolated methylene signal at δ 3.52 and 3.61 (H-21). The observation of ester carbonyl and oxymethylene resonances at δ 175.2 and δ 69.4, respectively, are consistent with the presence of the methyl ester and hydroxymethyl groups. The broad singlet at δ 7.98 is attributed to the indolic NH associated with the disubstituted indole moiety. This is based on the observed NOE between NH and H-12 (δ 7.05) of the disubstituted indole unit which also allowed assignment of the aromatic AB doublets to H-11 and H-12. Since the molecular formula showed the presence of four oxygen atoms and three have already been accounted for, the remaining oxygen must be due to a phenolic OH associated with the disubstituted indole moiety. This was further supported by acetylation (Ac2O/DMAP), which yielded a diacetate derivative 4 (δH 1.72 and 1.98; δC 20.2, 169.0, 209.3, 170.8; see Section 3).

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Both leucofoline (1) and leuconoline (2) showed weak cytotoxicity towards drug-sensitive as well as vincristine-resistant (VJ300) KB cells (IC50 values of 12.9 and 13.2 µg/mL for 1 against KB and KB/VJ300, respectively; and 11.5 and 12.2 µg/mL for 2 against KB and KB/VJ300, respectively).

3. Experimental

3.1. General

Optical rotations were determined on a Jasco P-1020 digital polarimeter. IR spectra were recorded on a Perkin–Elmer RX1 FT-IR spectrophotometer. UV spectra were obtained on a Shimadzu UV-3101PC spectrophotometer. 1H and 13C NMR spectra were recorded in CDCl3 using TMS as internal standard on a JEOL JNM-LA 400 and JNM-ECA 400 spectrometers at 400 and 100 MHz, respectively. EIMS and HREIMS were obtained at Organic Mass Spectrometry, Central Science Laboratory, University of Tasmania, Tasmania, Australia. ESIMS and HRESIMS were obtained on an Agilent 6530 Q-TOF mass spectrometer. X-ray diffraction analysis was carried out on a Bruker SMART APEX II CCD area detector system equipped with a graphite monochromator and a Mo Kα fine-focus sealed tube (λ = 0.71073 Å). All air-moisture-sensitive reactions were carried out under N2 in oven-dried glassware. CH2Cl2 was freshly distilled from CaH2, under N2. All other reagents were used without further purification.

3.2. Plant material

Details of collection, identification and deposition of plant materials have been reported previously (Gan et al., 2009a).

3.3. Extraction and isolation

Extraction of the ground stem-bark materials were carried out in the usual manner by partitioning the concentrated EtOH extract with dilute acid as has been described in detail elsewhere (Kam and Tan, 1990). Extraction was carried out on 13.8 kg of the stem-bark material. The alkaloids were isolated by initial column chromatography of the basic fraction on silica gel using CHCl3 with increasing proportions of MeOH, followed by rechromatography of the appropriate partially resolved fractions using preparative centrifugal TLC. Initial column chromatography of the basic fraction of the stem-bark extract provided three fractions. Subsequent preparative centrifugal TLC (silica gel, CHCl3/MeOH, 0.5% NH3) of fraction-2 furnished 1 (0.4 mg/kg) and 2 (2.9 mg/kg).

3.4. Characterization data

3.4.1. Leucofoline (1)

Light yellowish oil; [α]D +113 (c 0.28, CHCl3); UV (EtOH), λmax (log ε): 214 (4.26), 273 (3.82) nm; 1H and 13C NMR data, see Table 1; EIMS m/z 557 [MH]+; HRESIMS m/z: 557.3642 (calc. for C38H44N4 + H, 557.3644).

3.4.2. Leuconoline (2)

Light yellowish oil and subsequently light yellowish crystals from EtOH; m.p. 223–224 °C; [α]D +142 (c 0.49, CHCl3); UV (EtOH), λmax (log ε): 230 (4.72), 285 (4.21) nm; IR (dry film) νmax: 3401, 1713 cm−1; 1H and 13C NMR data, see Table 1; EIMS (probe) 70 eV, m/z (rel. int.): 646 [M]+ (100), 615 (12), 543 (12), 463 (10), 433 (3), 393 (6), 323 (4), 252 (45), 197 (7), 156 (4), 124 (15); HRESIMS m/z: 646.3510 (calc. for C38H44N4O4, 646.3519).

C-16′ (Kam et al., 1999b; Kam and Choo, 2006). This is also supported by the observed coupling constants for H-16' of 12 and 4.4 Hz which are consistent with an axial or α-oriented H-16' and diagnostic of eburnane alkaloids belonging to the eburnamine series, as opposed to those belonging to the diastereomeric isoeburnane (or epi-eburnamine) series (Kam et al., 1993; Kam and Choo, 2006; Kam and Lim, 2008).

Since suitable crystals of leuconoline (2) were obtained, an X-ray diffraction was also carried out which provided further confirmation of the structure deduced from analysis of the spectroscopic data (Fig. 4).

We recently reported the structure of the new bisindole alkaloid, leucohyphillinidine (7) (vide supra), which is constituted from the union of an eburnane and a vinylquinoline unit, and suggested a biogenetic pathway from an Aspidosperma precursor (Gan et al., 2009b). In view of an error involving the configuration of the precursor alkaloid 8, we now include a revised version of the pathway (Scheme 2), which incorporates the correct enantiomer of the Aspidosperma precursor.
3.5. Acetylation of leuconoline (2)

To a stirred solution of 2 (9.6 mg, 0.015 mmol), CH2Cl2 (2 mL) and 4-(dimethylamino)pyridine (DMAP, 4.5 mg, 0.037 mmol) was added dropwise acetic anhydride (7 μL, 0.075 mmol), and the mixture was stirred at room temperature for 2 h. The mixture was quenched with 10% Na2CO3 (5 mL) and extracted with CH2Cl2 (3 x 10 mL). The combined organic layers were dried (Na2SO4), the solvent evaporated in vacuo, and the residue purified by preparative centrifugal TLC (SiO2; Et2O/hexanes 1:1) to give 7.2 mg (67%) of the diacetate derivative 4 as light yellowish amorphous solids; m.p. >224 °C; [α]D +113 ([0.07, CHCl3]; UV (EtOH), λmax (log ε) 210 (4.46), 230 (4.63), 287 (4.08) nm; IR (dry film) vmax: 3378, 1746, 1717 cm−1; 1H NMR (CDCl3, 400 MHz): δ 0.96 (3H, t, J = 7 Hz, Me-18′), 1.20 (1H, m, H-15a), 1.42 (1H, m, H-14′a), 1.58 (3H, d, J = 7 Hz, H-18), 1.61 (1H, m, H-19′a), 1.63 (1H, m, H-15′b), 1.72 (3H, s, COMe), 1.83 (1H, m, H-14′b), 1.91 (1H, m, H-14a), 1.98 (3H, s, COMe), 2.14 (1H, m, H-19b), 2.23 (1H, m, H-17′a), 2.46 (1H, m, H-3′a), 2.50 (1H, m, H-17b), 2.59 (1H, m, H-3′b), 2.62 (1H, m, H-6′a), 2.63 (1H, m, H-14b), 2.89 (1H, m, H-5), 3.01 (1H, m, H-6′b), 3.03 (3H, s, CO2Me), 3.14 (1H, m, H-6a), 3.21 (1H, m, H-15), 3.29 (1H, td, J = 11 and 5 Hz, H-5′a), 3.38 (1H, dd, J = 13.6 and 6.3 Hz, H-5′b), 3.46 (1H, m, H-6b), 3.54 (1H, d, J = 17 Hz, H-21a), 3.65 (1H, d, J = 17 Hz, H-21b), 4.04 (1H, s, H-21′), 4.13 (1H, d, J = 10.5 Hz, H-17a), 4.28 (1H, d, J = 10.5 Hz, H-17b), 4.25 (1H, d, J = 12 Hz, H-3), 5.39 (1H, q, J = 7 Hz, H-19), 5.79 (1H, dd, J = 11 and 5.2 Hz, H-16′), 6.42 (1H, d, J = 8 Hz, H-12′), 6.68 (1H, t, J = 7.6 Hz, H-11′), 6.70 (1H, d, J = 9 Hz, H-11), 6.93 (1H, t, J = 7.6 Hz, H-10′), 7.08 (1H, d, J = 9 Hz, H-12), 7.39 (1H, d, J = 7.6 Hz, H-9′), 8.38 (1H, br s, NH); ESI-MS m/z 731 [MH]+; HRESIMS m/z: 731.3813 (calc. for C44H50N4O6 + H, 731.3803).

3.6. X-ray diffraction analysis of leuconoline (2)

A single crystal of 2 was obtained from EtOH; C40H46N4O4, Mw = 646.81, orthorhombic, space group P212121, a = 7.0778(2) Å, b = 13.0650(4) Å, c = 37.7977(12) Å; U = 3495.2(12) Å3. Z = 4. Dcalc = 1.229 g cm−3. The structure was solved by direct methods and refined by the least-square method. The final R value is 0.0644 (Rw = 0.1387) for 3119 reflections [I > 2σ(I)]. CCDC 770690 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

Cytotoxicity assays

Cytotoxicity assays were carried out following the procedure that has been described in detail previously (Kam et al., 1998a, 2004a).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2010.05.015.

References