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Aglairubine: Structure Revision of a Chemotaxonomically Interesting Bisamide in *Aglaia* (Meliaceae)

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Summary. The bisamide aglairubine was isolated from different *Aglaia* species. The original structure was revised on the basis of FDMS and 2D-NMR data. Since all isolates were obtained from species belonging to the section Amoora of the genus *Aglaia*, aglairubine might serve as a taxonomic marker.

Keywords. Aglairubine; Bisamide; Putrescine; *Aglaia*; Meliaceae.

Introduction

The formation of bisamides represents a typical chemical trend in the genus *Aglaia* of the family Meliaceae. They are mostly accumulated in the leaves [1–8]. Aglairubine was originally isolated from the leaves of *A. rubiginosa* (Hiern) C. M. Pannell and described as a putrescine bisamide linked with two different acid moieties: one nitrogen is linked with cinnamic acid, the second is included in a cyclic (lactamic) dihydropyrrolone ring [5]. In the course of our current screening for new characteristic constituents of the genus we recently isolated the same compound as a major constituent from young leaves of freshly germinated seedlings of *A. spectabilis* (Miq.) Jain and Bennet, collected in Khao Yai National Park (Thailand). Furthermore, small amounts were also isolated from *A. australiensis* C. M. Pannell and *A. meridionalis* C. M. Pannell, collected in Atherton Tableland, Queensland, Australia (*K. Teichmann*, Master thesis, in preparation). Since all four species are grouped together in the section Amoora [9], aglairubine may represent a characteristic chemical feature of this taxonomic group. Based on our extensive

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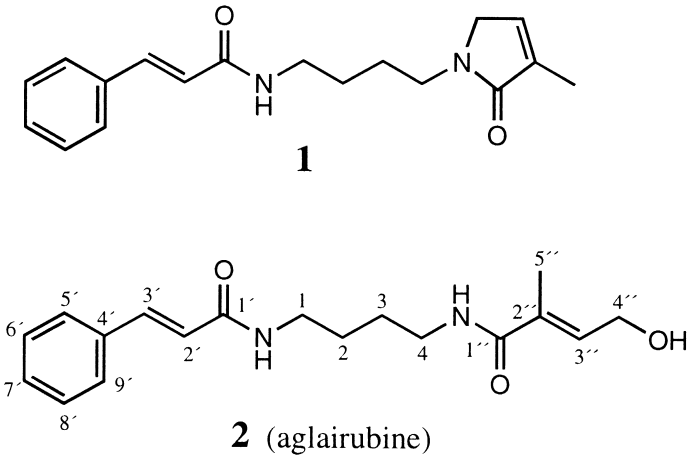


Scheme 1. Original (**1**) and revised (**2**) structure of aglairubine

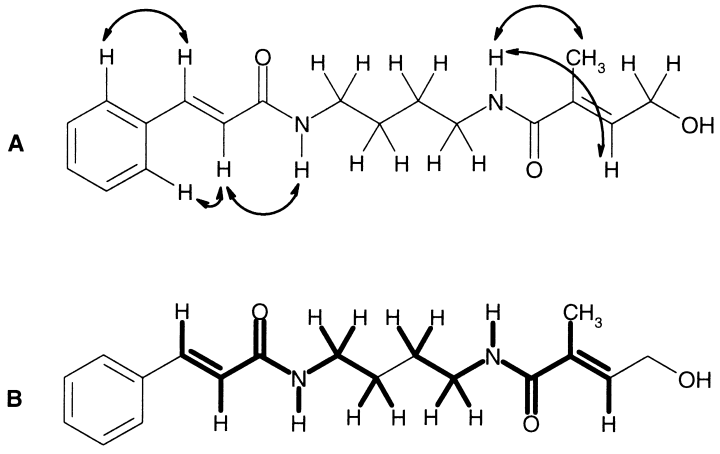
efforts into structure elucidation with 2D-NMR methods and FDMS, which were not used in the original structure determination [5], we had to revise the published structure (**1**) and to establish a new one (**2**) for aglairubine by replacing the dihydropyrrolone moiety by the amide of (*E*)-4-hydroxy-2-methyl-2-butenoic acid (Scheme 1).

Results and Discussion

The ^{1}H and ^{13}C NMR data sets measured in CD_3OD and the EIMS fragmentation pattern for the compound isolated from *A. spectabilis* seedlings were identical with the data of aglairubine listed in Ref. [5] and agreed perfectly with an authentic sample from *A. rubiginosa*[1]. However, the ^{1}H and ^{13}C chemical shift values of $\delta = 4.23$ and 59.5 ppm seemed rather high for a CH_2 group attached to nitrogen, even in an unsaturated lactam ring as in structure **1**. The corresponding literature data for N-butyl-3-methyl-2,5-dihydro-2-pyrrolone with an identical lactamic moiety are 3.83 and 50.7 ppm [10]. The experimental values for the methylene group in question seemed therefore better compatible with a neighbouring oxygen atom. A clear NOE between the methyl group and CH_2–N was also not compatible with the originally postulated structure **1**. An FDMS M^+ peak of $m/z = 316$ suggested a molecular formula of $C_{18}H_{24}N_2O_3$ and not $C_{18}H_{22}N_2O_2$ as derived from EIMS ($m/z = 298$) obtained for structure **1** [5]. The difference corresponds to one equivalent of H_2O which is obviously readily lost under EIMS conditions.

Structure **2** is also compatible with the NOE contact observed between the methyl group ($5''$-H_3) and the OCH_2 group ($4''$-H_2) corroborating the *cis* orientation of these groups (*E*-configuration). As a further proof for the revised structure **2** for aglairubine, the NMR spectra of both the isolated and the reference sample were recorded in *DMSO*-d_6 since the exchangeable NH and OH protons could not be detected in the original spectra measured in CD_3OD. The spectra of both samples were identical and, as expected, two NH and one OH signal could be

[1] The authentic sample was provided by the laboratory of *E. Saifah*



Scheme 2. Selected dipolar (**A**) and scalar (**B**) coupled networks of structural relevance for aglairubine (**2**)

identified in addition to the resonances observed in CD_3OD. The amide signals appeared as triplets, whereas the CH_2–OH signal was broadened due to chemical exchange with the bulk water. All observed COSY, NOESY, and long-range shift correlation data (HMBC), especially all NH and OH correlations, are fully compatible with constitution **2** for aglairubine (Scheme 2).

Experimental

General

HPLC: Separations and purity examinations were performed with a Hewlett-Packard HP 1090 II, UV diode array detection, column 290 × 4 mm (Spherisorb octadecyl silica (ODS), 5 μm), mobile phase MeOH (gradient 60–100%) in aqueous puffer (0.0015 *M* phosphoric acid, 0.00015 *M* tetrabutylammonium hydroxide, $pH = 3$), flow rate 1 cm^3/min. FDMS: Finnigan MAT 900S. NMR: The NMR experiments were performed on a wide-bore Bruker DRX 400 instrument equipped with either a tuneable broad band inverse probe tuned to ^{13}C (for the inverse detected gradient selected 2D experiments) or a dual $^1H/^{13}C$ probe for the 1D experiments. The sample concentration was 9 mg/0.7 cm^3 and 20 mg/0.7 cm^3 in the case of the isolated material and the reference material from Thailand, respectively. Measurements were performed both in CD_3OD and *DMSO*-d_6 to allow comparison with the published shift values. The spectra were either referenced to internal *TMS* (^1H, 0.00 ppm) or to the appropriate solvent signals (^{13}C, CD_3OD 49.0 ppm, *DMSO*-d_6 39.5 ppm). The sample temperature was kept at 300 K. Standard pulse sequence programs provided by the spectrometer manufacturer were used. The HMBC experiments were optimized for a long-range coupling constant of 8 Hz.

Plant material

A. australiensis and *A. meridionalis* were collected in Atherton Tableland, Queensland, Australia, and *A. spectabilis* in Khao Yai National Park, Thailand. Voucher specimens are deposited at the herbarium of the Institute of Botany, University of Vienna (WU).

Extraction and isolation

Air dried leaves (2 g) from seedlings of *A. spectabilis* were homogenized and extracted twice with MeOH for 3 days at room temperature. The concentrated residue, obtained on removal of the solvent

from the filtered extract under reduced pressure, was partitioned between $CHCl_3$ and H_2O. The organic phase was evaporated to dryness (109 mg) and roughly separated by column chromatography (Merck Si gel 60, 0.2–0.5 mm) using solvent mixtures with increasing polarity (hexane, EtOAc, MeOH). CC-Fractions eluted with 50% MeOH in EtOAc and 100% MeOH were combined (45 mg) and further separated by MPLC employing a stepwise gradient from 100% EtOAc to 50% MeOH in EtOAc (400 × 40 mm, Merck Lichroprep silica 60, 25–40 µm, UV detection, 254 nm). Aglairubine was eluted in a fraction of 10% MeOH in EtOAc (27 mg) and was finally purified by preparative TLC (Merck, Si gel 60, 0.5 mm, CH_2Cl_2 : EtOAc : MeOH = 70 : 23 : 7) to give 9 mg pure compound.

(E,E)-N_1-Cinnamoyl-N_2-(4-hydroxy-2-methyl-2-butenoyl)-putrescine
(**2**, aglairubine; $C_{18}H_{24}N_2O_3$)

^{1}H NMR (*DMSO*-d_6, 400 MHz): $\delta = 8.07$ (t, 1H, $J = 5.5$ Hz, 1-NH), 7.80 (t, 1H, $J = 5.5$ Hz, 4-NH), 7.55 (dd, 2H, $J = 7.8$ and 1.5 Hz, 5′/9′-H), 7.41 (d, 1H, $J = 15.8$ Hz, 3′-H), 7.33–7.45 (m, 3H, 6′/8′ and 7′-H), 6.61 (d, 1H, $J = 15.8$ Hz, 2′-H), 6.27 (tq, 1H, $J = 5.8$ and 1.2 Hz, 3″-H), 4.79 (t, 1H, $J = 5.4$ Hz, 4″-OH), 4.08 (dd, 2H, $J = 5.8$ and 5.4 Hz, 4″-H_2), 3.18 (m, 2H, 1-H_2), 3.12 (m, 2H, 4-H_2), 1.71 (s, 3H, 5″-H_3), 1.46 (m, 4H, 2-H_2 and 3-H_2) ppm; ^{13}C NMR (*DMSO*-d_6, 100 MHz): $\delta = 168.0$ (s, C-1″), 164.7 (s, C-1′), 138.3 (d, C-3′), 134.9 (s, C-4′), 134.8 (d, C-3″), 130.4 (s, C-2″), 129.3 (d, C-7′), 128.8 (d, C-6′/8′), 127.4 (d, C-5′/9′), 122.3 (d, C-2′), 57.9 (t, C-4″), 12.7 (q, C-5″), 38.6 and 38.4 (2 × t, C-1 and C-4), 26.7 and 26.6 (2 × t, C-2 and C-3) ppm; NOESY (H → H): 1-NH → 1, 2, 2′; 1 → 1-NH; 4 → 4-NH; 4-NH → 3, 2″, 5″; 2′ → 5′/9′, 1-NH; 5′/9 → 2′; 3″ → 4″, 4-NH, (4″-OH), 4″ → 3″, 4″-OH, 5″; 4″-OH → 4″, (3″); 5″ → 4″, 4-NH; HMBC (C → H): 1 → 2, 3; 2 → 1, 4; 3 → 1, 4; 4 → 2, 3; 1′ → 1, 1-NH, 2′, 3′; 2′ → 3′; 3′ → 5′/9′; 4′ → 2′, 6′/8′; 5′/9′ → 6′/8′; 6′/8′ → 5′/9′; 1″ → 4, 4-NH, 3″, 5″; 2″ → 4″, 5″; 3″ → 4″, (4″-OH), 5″; 4″ → (4″–OH); 5″ → 3″ (weak cross peaks in parentheses); FDMS: $m/z = 316$ (M^+).

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