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Short Report

Alkaloid and other Chemical Constituents from *Psychotria stachyoides* Benth.

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Os extratos orgânicos das folhas e raízes de *Psychotria stachyoides* forneceram o novo alcaloide indólico monoterpênico glicosilado N-desmetil-correantosida, além de bizantionosida B, α-amirina, éter metílico da alizarina, rubiadina, escopoletina, ácido barbinévico e uma mistura de β-sitosterol e estigmasterol glicosilados. A caracterização estrutural dos compostos isolados foi estabelecida com base na espectroscopia no infravermelho (IR), espectrometria de massa (MS) e particularmente ressonância magnética nuclear (RMN) 1D e 2D.

The organic extracts of leaves and roots of *Psychotria stachyoides* provided the new glucoside monoterpenoid indole alkaloid N-demethylcorreantoside, besides bizantionoside B, α-amyrin, alizarine methyl-ether, rubiadine, scopoletin, barbinevic acid and a mixture of β-sitosterol and stigmasterol glucosides. The structural characterization of the isolates was established based on infrared spectroscopy (IR), mass spectrometry (MS) and, particularly, 1D and 2D nuclear magnetic resonance (NMR).

Keywords: monoterpene indole alkaloid, coumarins, naphthoquinones, triterpenes, *Psychotria stachyoides*

Introduction

Indole alkaloids represent the biggest single class of all alkaloids from plants. The major groups under investigation are the ones with monoterpene (C_{10}) or *nor*-monoterpene (C_9) moieties joined to the tryptamine, which are distributed on a wide range of different structures in the Apocynaceae, Loganiaceae, Nyssaceae and Rubiaceae families. The genus *Psychotria* (Rubiaceae) comprises more than 1000 species distributed in tropical regions worldwide. This genus is taxonomically complex, and is particularly characterized as a prolific source of bioactive polyindole and monoterpenoid indole alkaloids. Previous phytochemical studies of species of *Psychotria* from the Southern Brazil have revealed the remarkable presence of glycosylated indole monoterpenoid alkaloids.[1]

As part of the investigative efforts to find alkaloids from Rubiaceae species of the Northeastern Brazil flora, *Psychotria stachyoides* (subgroup *Heteropsychotria*), a shrub growing predominantly in tropical and sub-tropical forests from Brazil (where it is popularly named as "erva-d'anta"),[2] has been investigated. This work reports the isolation and structural characterization of the new glucosilated monoterpene indole alkaloid named as N-demethylcorreantoside (**1**) from the leaves of *P. stachyoides*, in addition to the known bizantionoside B and α-amyrin.[3,4] Chemical investigation of the roots yielded alizarin methyl-ether,[5] rubiadine,[6] scopoletin,[7] barbinevic acid[8] and a mixture of β-sitosterol and stigmasterol glucosides.[9]

Results and Discussion

The molecular formula of compound **1** was established by the positive mode HRESIMS (electrospray ionization mass spectrometry) peak at *m/z* 499.2039 ($[M + H]^+$ (497.9654 + 1.00783 calcd. for $C_{26}H_{30}N_2O_8$ + H). The IR spectrum implied the presence of hydroxyl (3414 cm^{-1}) and lactam (1675 cm^{-1}) functionalities. From the ^1H NMR spectrum, a tetrahydro-β-carboline system was defined by

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the typical aromatic signals at δ 7.22 (td, *J* 7.4 and 1.0 Hz, H-10), 7.26 (td, *J* 7.4 and 1.3 Hz, H-11), 7.45 (dd, *J* 7.4 and 1.3 Hz, H-9), 8.15 (dd, *J* 7.4 and 1.0 Hz, H-12), besides the two methylenes at δ 3.10 (t, *J* 5.5 Hz, 2H-5) and 2.70 (t, *J* 5.4 Hz, 2H-6).[10] The ^{1}H NMR spectrum also showed signals relative to an iridoid unit related to a secologanin moiety due to the signals of the olefinic hydrogen at δ 5.85 (ddd, *J* 17.6, 10.0 and 7.6 Hz, H-19), the vinylidene group at δ 5.20 (d, *J* 10.0 Hz, H-18a) and 5.23 (d, *J* 17.6 Hz, H-18b), and the methines at δ 2.64 (m, H-20 and H-15) and 5.74 (d, *J* 8.7 Hz, H-21).[11] The sugar moiety was assigned as an O-β-D-glucopyranose on the basis of the larger *J* value for the anomeric proton at δ 4.82 (d, *J* 8.2 Hz, H-1').

The presence of the monoterpenoid unit was confirmed by analysis of the ^{1}H, ^{1}H COSY NMR data. This spectrum exhibited correlations for the hydrogens at δ 5.74 (H-21) and 2.64 (H-20), which in turn, showed correlation to the hydrogen at δ 5.85 (H-19). The strong correlations observed for the methine hydrogen at δ 4.20 (H-3) and the methylene group at δ 1.98 (H-14α) and 2.10 (H-14β) suggested that the secologanin unit was attached to the C-3 carbon, as reported for other similar monoterpenoid alkaloids.[10] This suggestion was supported by the ^{13}C NMR spectrum along with the distortionless enhancement by polarization transfer (DEPT 135), that revealed 26 carbons atoms corresponding to three methylenes, one oxymethylene and one vinylidene, three methines, two hemiketal methynes and four oxymethines, six monohydrogenated sp^2 carbons, and six non-hydrogenated carbons. However, the confirmation of the structure of **1** was supported by the HMQC and HMBC (heteronuclear multiple quantum coherence and heteronuclear multiple bond coherence, respectively) experiments, that revealed the unequivocal assignments of its NMR data. Further evidences of a tetrahydro-β-carboline skeleton were possible by the observed long-range correlations in the HMBC spectrum, that showed the proton at δ 3.10 (2 H-5) correlated to the carbons at δ 50.6 (C-3) and 117.0 (C-7), while the proton at δ 2.70 (2 H-6) showed correlations to the carbon at δ 117.0 (C-7) and 136.0 (C-2). In particular, the linkage of the methylene carbon C-14 with C-3 was established by the key correlations of the methylene hydrogens at δ 1.98 and 2.10 (2H-14) with the carbons at δ 136.0 (C-2) and 50.6 (C-3). The glucosyl moiety positioned at C-21, was supported by the correlation between the anomeric hydrogen at δ 4.82 (H-1') and the carbon at δ 97.5 (C-21). The structure of **1** was finally elucidated by comparing the value of the deshielded signal relative to H-12 (δ 8.15), to those reported for typical monoterpenoid indole alkaloids (δ *ca.* 7.6).[12] This chemical shift difference to a more deshielded position provided the clue for the determination of a seven member lactam ring derived from the intramolecular condensation of the indole nitrogen (N-1) with the original carbonyl of the methyl ester of secologanin. Comparison of these spectral features with those related to correantoside, a monoterpene indole alkaloid previously isolated from *P. correae,* showed high similarity,[12] with exception of the N-methyl group at N-1, present in correantoside but missing in **1**.

The relative stereochemistry of **1** was established by the nuclear Overhauser spectroscopy (NOESY) experiment. In particular by the diagnostic nuclear Overhauser effect (nOe) cross-peaks observed for the hydrogen at δ 1.98 (H-14α) with the hydrogens at δ 2.64 (H-20), and for the hydrogen at δ 2.10 (H-14β) with the hydrogen at δ 5.74 (H-21), in agreement with the relative stereochemistry of correantoside.[12]

From the above evidences, compound **1** was identified and named as the new N-demethylcorreantoside. The chemical structure and the observed key nOe correlations for compound **1** are presented in Figure 1.

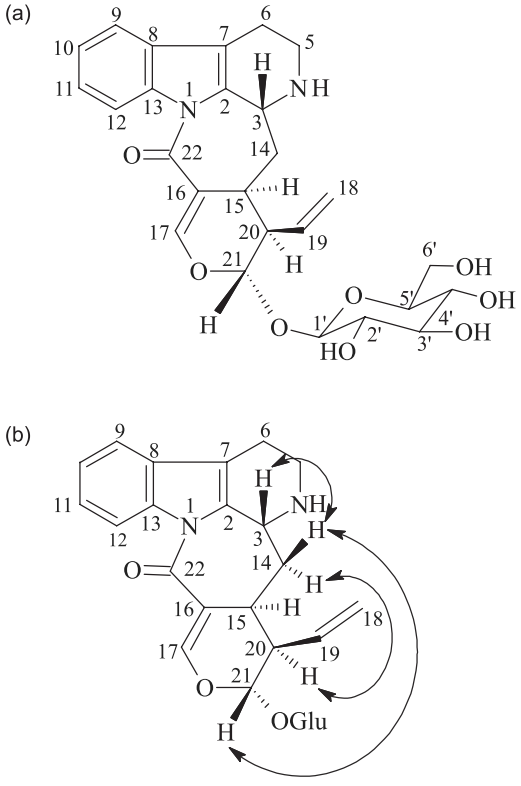

Figure 1. (a) Chemical structure and (b) observed key nOe correlations for compound **1**.

Experimental

General experimental procedures

Infrared (IR) spectra were recorded on a Perkin-Elmer FT-IR 1000 spectrometer (Waltham, USA) using KBr

pellets. The NMR spectra were performed on a Bruker Avance DRX 500 spectrometer, equipped with an inverse detection probehead and z-gradient accessory working at 500.13 (^{1}H) and at 125.77 MHz (^{13}C), respectively. All pulse sequences are standard in the Bruker XWIN-NMR software, and all experiments were conducted at room temperature. The samples were dissolved in CD_3OD (0.6 mL) and transferred to 5 mm tubes. The ^{1}H and ^{13}C chemical shifts are expressed in the δ scale and were referenced to residual CH_3OH at δ 3.31 for proton and at δ 49.1 for carbon. High resolution mass spectra (HRMS) were recorded on an UltrOTOF-Q mass spectrometer (LC-IP-TOF model 225-07100-34-SHIMADZU) either by positive or negative ionization modes of the ESI source. High-performance liquid chromatography (HPLC) analyses were performed on a Shimadzu chromatographer (Japan) equipped with a ternary pump (Shimadzu LC-20AT) and UV detector (Shimadzu SPD-M20A), using Phenomenex RP-18 columns (analytical: 250 × 4.6 mm, 5 μm; semi-preparative: 250 × 10 mm, 10 μm). HPLC grade acetonitrile was purchased from Tedia Co. (São Paulo State, Brazil) and the HPLC grade water was obtained by a Milli-Q purification system (Millipore, Bedford, USA). Column chromatography was performed either over silica gel 60 (EMD, 70-230 mesh) or Sephadex LH-20 (Amersham Pharmacia Biotech, Uppsala, Sweden). Thin layer chromatography (TLC) was performed on precoated silica gel aluminum sheets (Merck) and the compounds were visualized by UV detection and by spraying with Dragendorff reagent or vanillin/perchloric acid/EtOH solution, followed by heating.

Plant material

Leaves and roots of *Psychotria stachyoides* Benth. were collected at the Pacoti County (Ceará State, Northeast of Brazil). Voucher specimens (# 31674) were deposited at the Herbário Prisco Bezerra (EAC) and identified by Dr. Piero Giuseppe Delpret from the Institut de Recherche pour le Développement-IRD, UMR AMAP, Montpellier, France.

Extraction and isolation

Dried leaves of *P. stachyoides* (2.7 kg) were exhaustively extracted with EtOH (3 × 9.0 L) at room temperature. Evaporation of the solvent under vacuum yielded the ethanol extract (130.0 g). The ethanol extract was treated with 10% aqueous HCl and the acid solution was extracted with $CHCl_3$ (3 × 150 mL). The aqueous phase was basified with NH_4OH to pH 9-10 and extracted with EtOAc (3 × 150 mL). The organic fractions were combined and the solvent was evaporated to yield the alkaloid fraction (2.2 g). The alkaloid fraction (2.2 g) was chromatographed on Sephadex LH-20 by elution with MeOH to afford fourty two fractions (20 mL), that were combined into five resulting fractions after TLC analysis. Flash chromatography of fraction (2) (0.43 g) using CH_2Cl_2/MeOH/NH_4OH (95:4:1) as eluent with increasing polarity yielded thirty one fractions (20 mL), which were pooled together in to four resulting sub-fractions according to TLC analysis. The sub-fraction (2)(4) (47.4 mg) was submitted to semi-preparative HPLC with a C_{18} column and using CH_3CN-H_2O (7:3) TFA 0.2% system as eluent, to obtain **1** (4.9 mg) and bizantionoside B (6.3 mg). Successive flash chromatography from the non-alkaloidal fraction (3.0 g) by elution with hexane/EtOAc (9:1) yielded α-amyrin (37.0 mg).

The EtOH extract of the roots (50.4 g) was redissolved in a mixture of MeOH:H_2O (1:1 v/v) and submitted to liquid-liquid partition with hexane, CH_2Cl_2, EtOAc and n-BuOH to give the correspondent four fractions. The hexane fraction (3.9 g) was re-chromatographed on Si gel by elution with hexane/EtOAc mixtures with increasing polarity to afford eighteen sub-fractions (20 mL), these fractions were combined into eleven sub-fractions according to TLC analysis. Chromatography of fraction (5) (210.0 mg) by elution with hexane/EtOAc (3:1) yielded rubiadine (4.2 mg) and alizarin methyl ether (7.1 mg), respectively. The CH_2Cl_2 fraction (2.6 g) was re-chromatographed on Si gel by elution with CH_2Cl_2/MeOH mixtures with increasing polarity, to give ninety two sub-fractions (20 mL), which were combined in nine resulting fractions according to TLC analysis. Fraction (2) yielded scopoletin (11.0 mg) and (4) yielded a mixture of β-sitosterol and stigmasterol glucosides (12.4 mg). Fraction (9) was re-chromatographed on Si gel by elution with hexane/EtOAc mixture with increasing polarity to yield barbinevic acid (20.8 mg).

*N-Demethylcorreantoside (**1**)*

Brown solid, mp 200.6-202.8 °C; $[\alpha]_D^{20}$ −173° (*c* 0.075, MeOH); IR (KBr) ν_{max}/cm^{-1}: 3414 (O–H), 1675 (C=O), 1454, 1376, 1201,1166; ^{1}H NMR (500 MHz, CD_3OD): 4.20 (dd, *J* 7.3 and 3.3 Hz, H-3), 3.10 (t, *J* 5.5 Hz, H-5), 2.70 (t, *J* 5.5 Hz, H-6), 7.45 (dd, *J* 7.4 and 1,3 Hz, H-9), 7.22 (td, *J* 7.4 and 1.0 Hz, H-10), 7.26 (td, *J* 7.4 and 1.3 Hz, H-11), 8.15 (dd, *J* 7.4 and 1.0 Hz, H-12), 1.98 (m, H-14β), 2.10 (m, H-14α), 2.64 (m, H-15), 7.71 (s, H-17), 5.20 (d, *J* 10.0 Hz, H-18b), 5.23 (d, *J* 17.6 Hz, H-18a), 5.85 (ddd, *J* 17.6, 10.0 and 7.6 Hz, H-19), 2.64 (m, H-20), 5.74 (d, *J* 8.7 Hz, H-21), 4.82 (d, *J* 8.2 Hz, H-1'), 3.23 (dd, *J* 9.1 and 8.2 Hz, H-2'), 3.41 (t, *J* 9.1 Hz, H-3'), 3,.30 (t, *J* 9.1 Hz, H-4'), 3.32 (m, H-5'), 3.70 (dd, *J* 11.9 and 6.2 Hz, H-6'), 3.93 (dd, *J* 11.9 and 1.9 Hz, H-6'); ^{13}C NMR (125 MHz, CD_3OD): 136.0

(C-2), 50.6 (C-3), 40.0 (C-5), 23.2 (C-6), 117.0 (C-7), 131.0 (C-8), 119.2 (C-9), 124.4 (C-10), 125.5 (C-11), 116.4 (C-12), 137.3 (C-13), 36.7 (C-14), 35.7 (C-15), 112.7 (C-16), 155.6 (C-17), 119.3 (C-18), 135.2 (C-19), 45.6 (C-20), 97.5 (C-21), 168.6 (C-22), 100.7 (C-1'), 74.9 (C-2'), 78.7 (C-3'), 71.8 (C-4'), 78.2 (C-5'), 63.1 (C-6'); HRESIMS positive ions at *m/z* 499.2039 $[M + H]^+$ (calcd. for $C_{26}H_{30}N_2O_8$: *m/z* 497.9654).

Supplementary Information

Supplementary data associated with this paper are available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgements

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Supplementary Information

Alkaloid and other Chemical Constituents from *Psychotria stachyoides* Benth.

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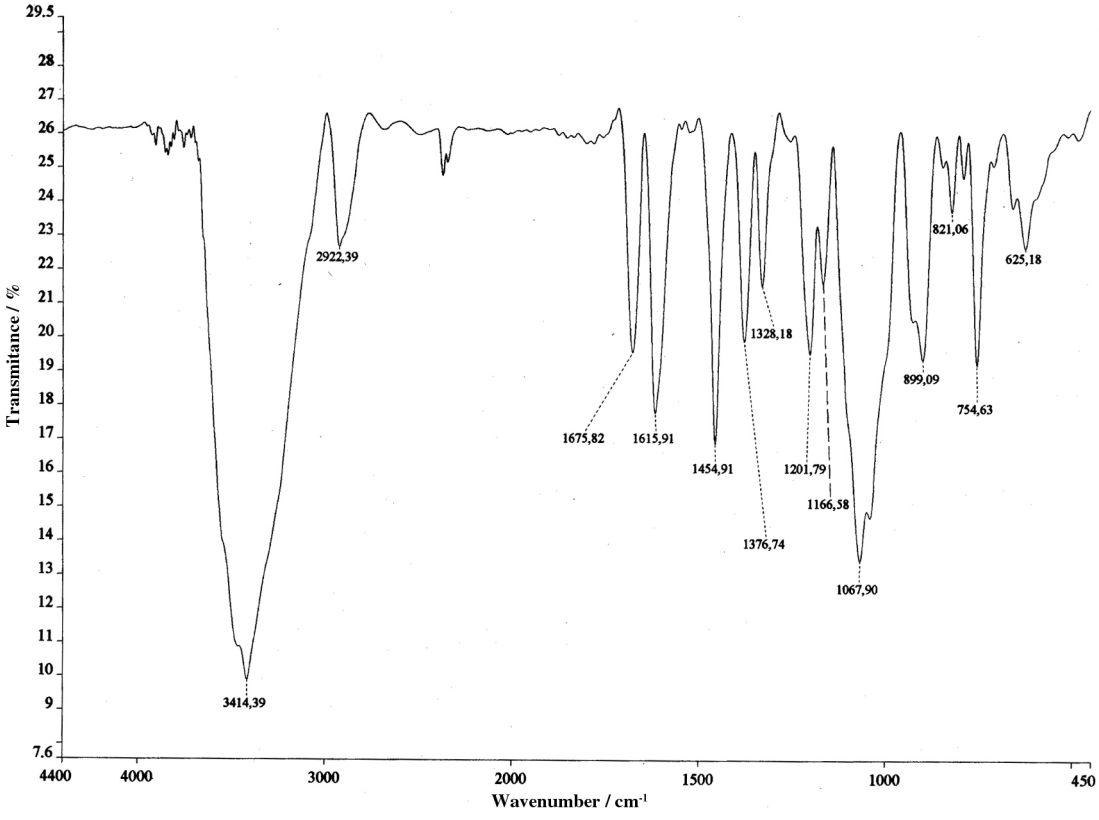

Figure S1. Infrared spectrum of **1** (KBr pellets).

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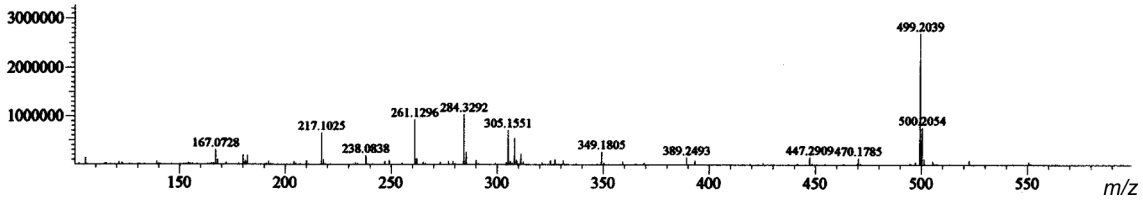

Figure S2. High resolution electrospray ionization mass spectrum of **1**.

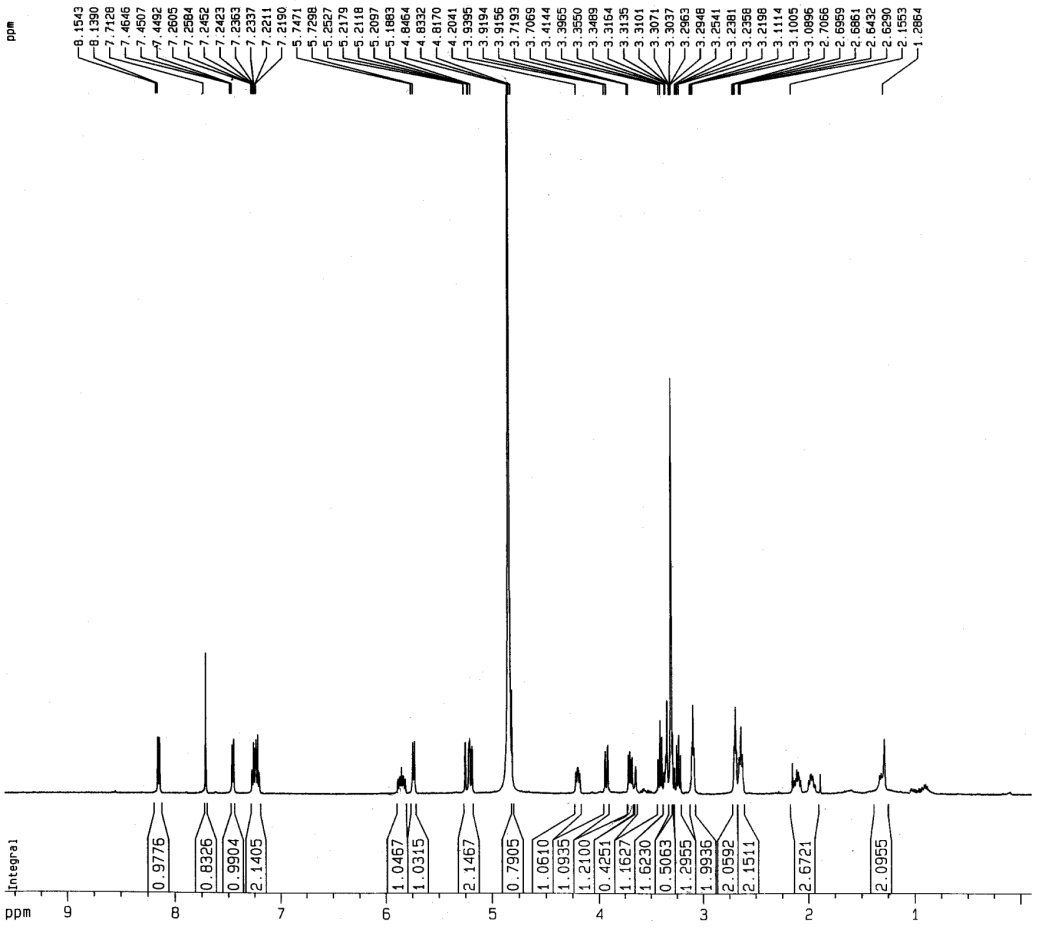

Figure S3. ^{1}H NMR spectrum of **1** (MeOD, 500 MHz).

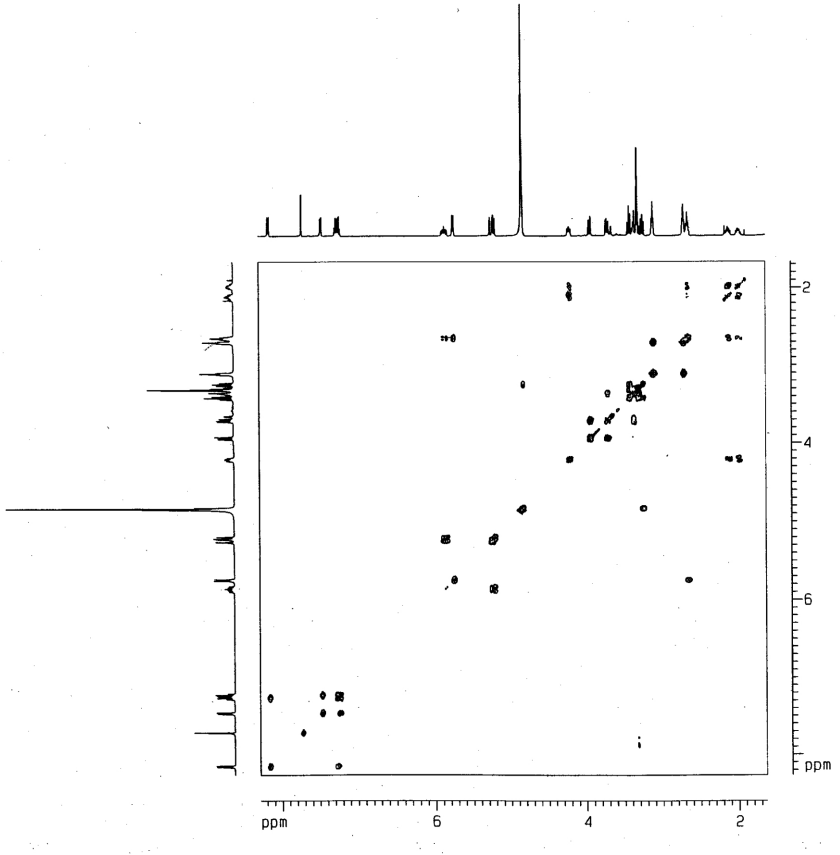

Figure S4. ^{1}H, ^{1}H COSY NMR spectrum of **1** (CD_3OD, 500 × 500 MHz).

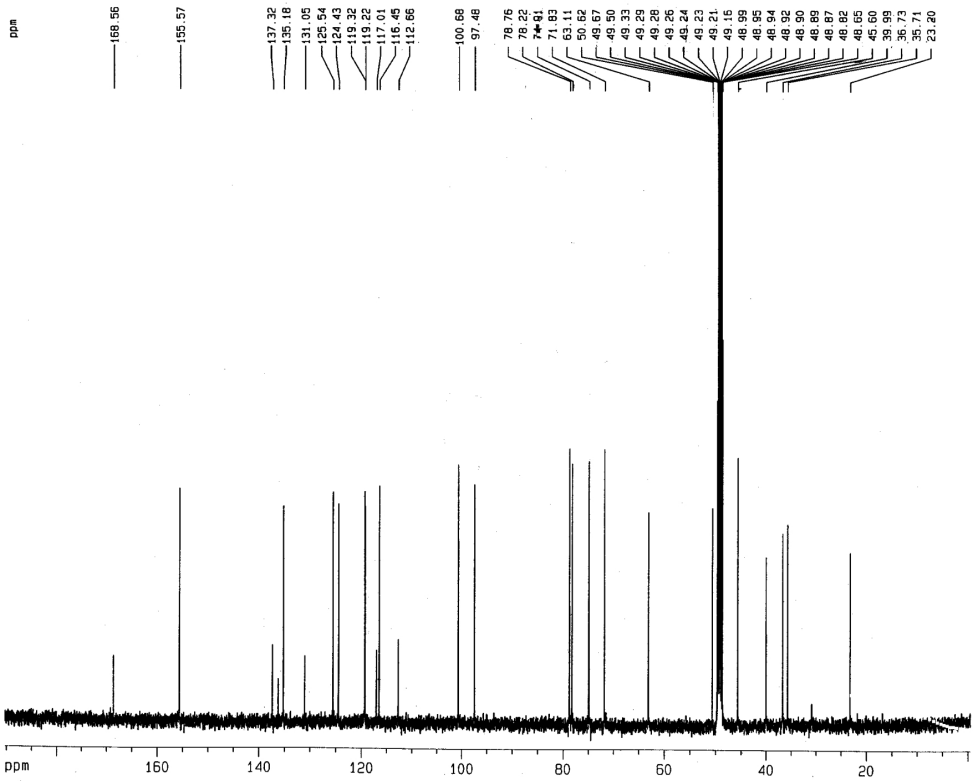

Figure S5. ^{13}C NMR spectrum of **1** (CD_3OD, 125 MHz).

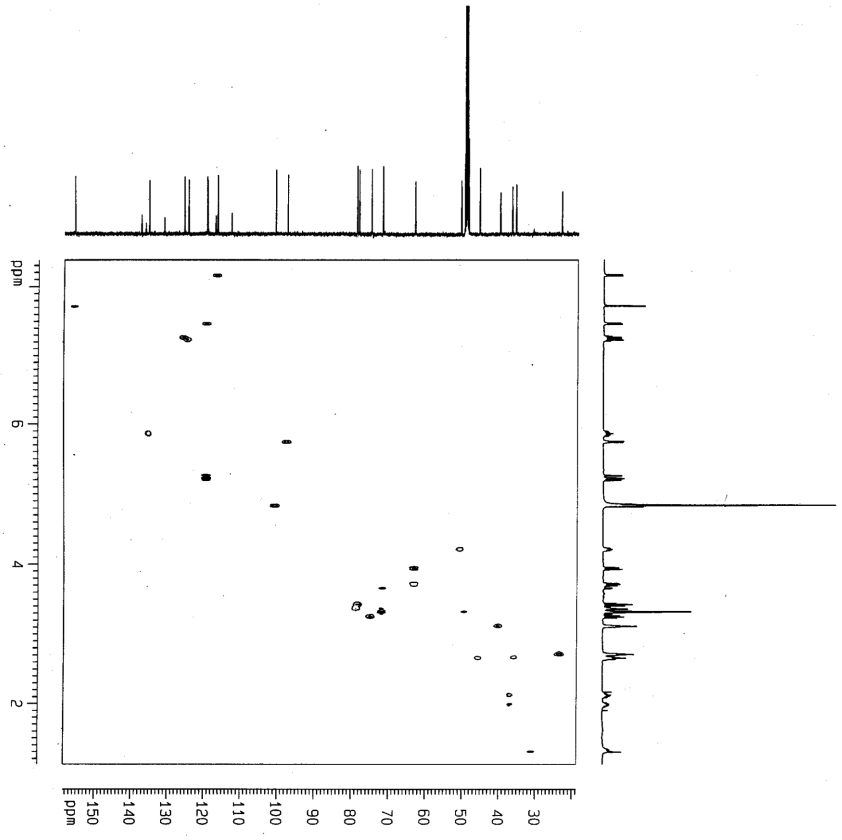

Figure S6. ^{1}H, ^{13}C HSQC-NMR spectrum of **1** (CD_3OD, 500 × 125 MHz).

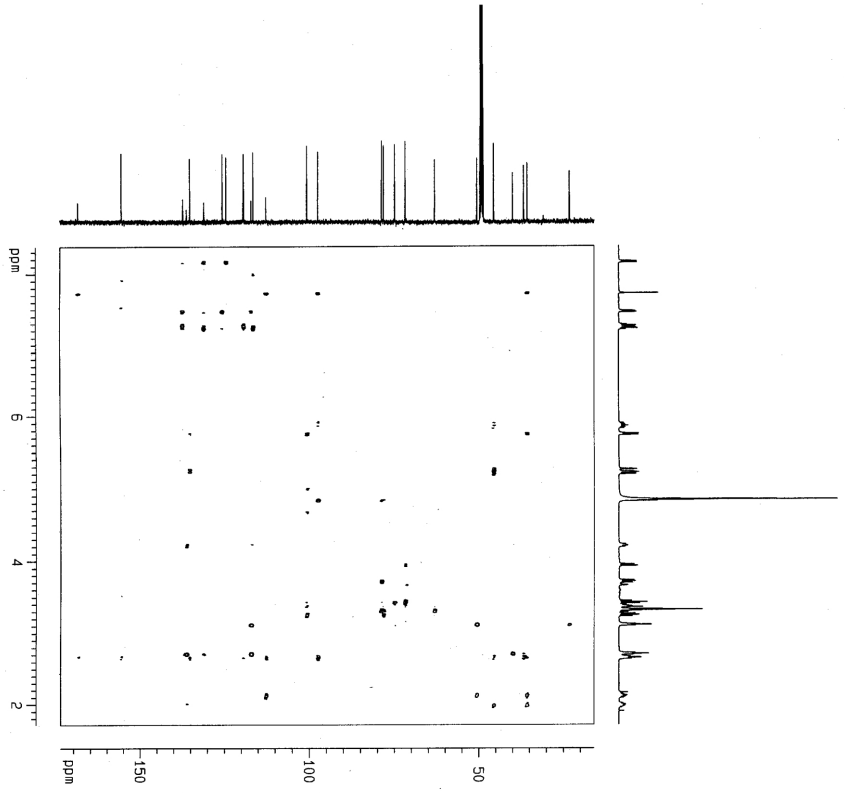

Figure S7. ^{1}H, ^{13}C HMBC-NMR spectrum of **1** (CD_3OD, 500 × 125 MHz).

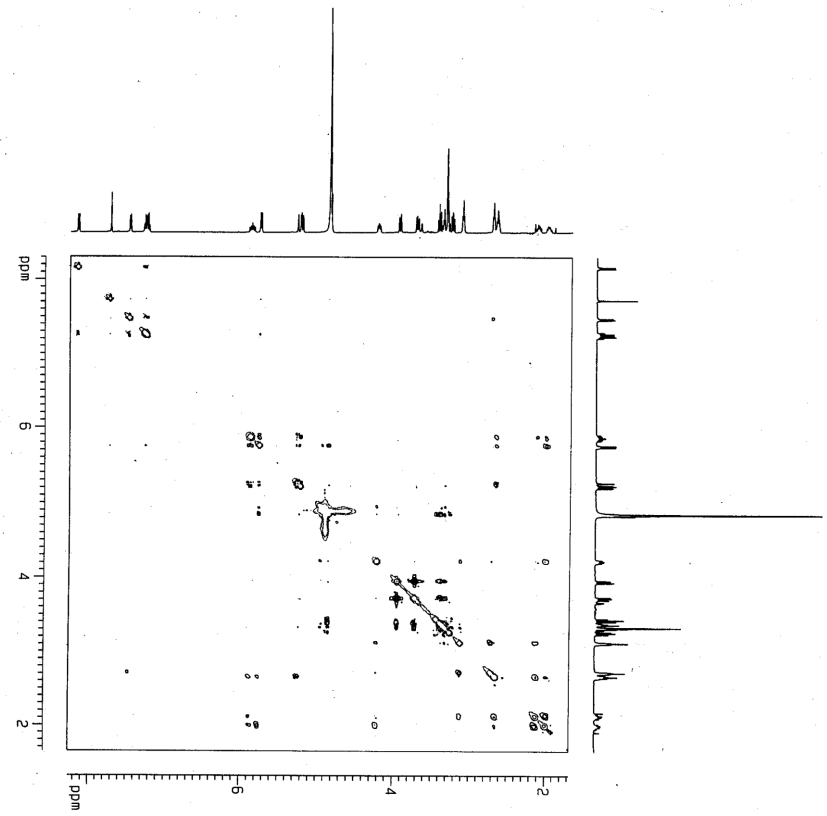

Figure S8. NOESY spectrum de **1**.