Supporting Information

Nigricanosides A and B, Antimitotic Glycolipids Isolated from the Green Alga *Avrainvillea Nigrans* Collected in Dominica

David. E. Williams[†], Christopher M. Sturgeon[¶], Michel Roberge[¶]*, and Raymond J. Andersen[†]*

Departments of Chemistry and Earth & Ocean Sciences, University of British Columbia, Vancouver, B. C., CANADA, V6T 1Z1; Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, B. C., CANADA, V6T 1Z3

Table of contents

Experimental Section

General Experimental Procedures. Optical rotations were measured using a Jasco P-1010 Polarimeter with sodium light (589 nm). The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV-600 spectrometer with a 5 mm CPTCI cryoprobe. ^{1}H chemical shifts are referenced to the residual C_6D_6 signal (δ 7.15 ppm) and ^{13}C chemical shifts are referenced to the C_6D_6 solvent peak (δ 128.0 ppm). Low and high resolution ESI-QIT-MS were recorded on a Bruker-Hewlett Packard 1100 Esquire–LC system mass spectrometer. Merck Type 5554 silica gel plates and Whatman MKC18F plates were used for analytical thin layer chromatography. Reversed-phase HPLC purifications were performed on a Waters 600E System Controller liquid chromatography attached to a Waters 996 Photodiode Array Detector. All solvents used for HPLC were Fisher HPLC grade.

Algal Material. Specimens of the green algae *Avrainvillea nigricans* Ducaisne were collected by hand using SCUBA in Prince Rupert Bay, Portsmouth, Commonwealth of Dominica at a depth of 25 m. The algal specimens were immediately frozen and transported frozen to Vancouver, Canada. A voucher specimen (ref. no. 04-307) is deposited at the Department of Earth and Ocean Sciences, University of British Columbia, Vancouver, Canada.

Extraction of *Avrainvillea nigricans* and Isolation of Nigricanoside A and B Dimethyl Esters (3 and 4). The thawed *Avrainvillea nigricans* (wet weight: 27.8 kg, dried extracted weight: 3.859 kg) was cut into small pieces, immersed in and subsequently extracted repeatedly with MeOH (3 x 30 L) at room temperature. The combined MeOH extracts were concentrated *in vacuo* and the resultant green oil was then partitioned between EtOAc (4 x 2000 mL) and H_2O (5.0 L). The combined EtOAc extract was evaporated to dryness and then extracted between hexanes (4 x 800 mL) and 4:1 MeOH/H_2O (2500 mL). The ratio of the methanolic extract was adjusted to 2:1 MeOH/H_2O (for a total of 3000 mL) and extracted with CH_2Cl_2 (4 x 1000 mL). The CH_2Cl_2 extracts were combined and evaporated to dryness to give 17.1 g of a potently active antimitotic amorphous green solid. To facilitate the ease of isolation the CH_2Cl_2 extract was methylated at this stage. Hence, the active material was treated with diazomethane that was generated *in-situ* by the addition of 30 mL of 2.0 M trimethylsilyldiazomethane in hexanes to 30 mL of anhydrous MeOH in 80 mL of C_6H_6. The reaction mixture was left stirring for 16 hours at room temperature. After evaporation of the reagents the sample was fractionated with Si gel flash chromatography employing a step gradient from 95:5 hexanes/EtOAc to MeOH. The fraction (750.7 mg) eluting with 1:3 MeOH/EtOAc elicited biological activity and was further fractionated on Sephadex LH-20 using 4:1 MeOH/CH_2Cl_2 as eluent. An early eluting potently active antimitotic fraction (206.0 mg) was obtained that was fractionated using reversed-phase Si gel flash chromatography employing a step gradient from 1:1 MeOH/H_2O to MeOH with a final CH_2Cl_2 wash. A 49.1 mg fraction, eluting with 3:2-4:1 MeOH/H_2O, elicited activity. Pure nigricanoside A dimethyl ester (**4**) (0.8 mg) and nigricanoside B dimethyl ester (**4**) (0.4 mg) were obtained from this mixture via C_{18} reversed-phase HPLC using a CSC-Inertsil 150A/ODS2, 5 µm 25 x 0.94 cm column, with 9:11 MeCN/H_2O as eluent.

Nigricanoside A Dimethyl Ester (**3**): Isolated as a clear oil; $[\alpha]^{25}_{D}$ -42 (c 0.24, CH_2Cl_2); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; positive ion HRESIMS $[M+Na]^+$ *m/z* 923.5323 (calcd for $C_{47}H_{80}O_{16}Na$, 923.5344).

Nigricanoside B Dimethyl Ester (**4**): Isolated as a clear oil; $[\alpha]^{25}_{D}$ -34 (c 0.20, CH_2Cl_2); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; positive ion HRESIMS $[M+Na]^+$ *m/z* 921.5186 (calcd for $C_{47}H_{78}O_{16}Na$, 921.5188).

Preparation of Perhydronigricanoside Dimethyl Ester: Nigricanoside B dimethyl ester (**4**) (0.4 mg) was dissolved in EtOH (0.5 mL) and reduced with H_2 gas on palladium/charcoal 10% catalyst with stirring overnight at rt. The reaction mixture was filtered and concentrated *in vacuo*.

Hydronigricanoside Dimethyl Ester: Isolated as a clear oil; positive ion HRESIMS $[M+Na]^+$ *m/z* 933.6126 (calcd for $C_{47}H_{90}O_{16}Na$, 933.6127).

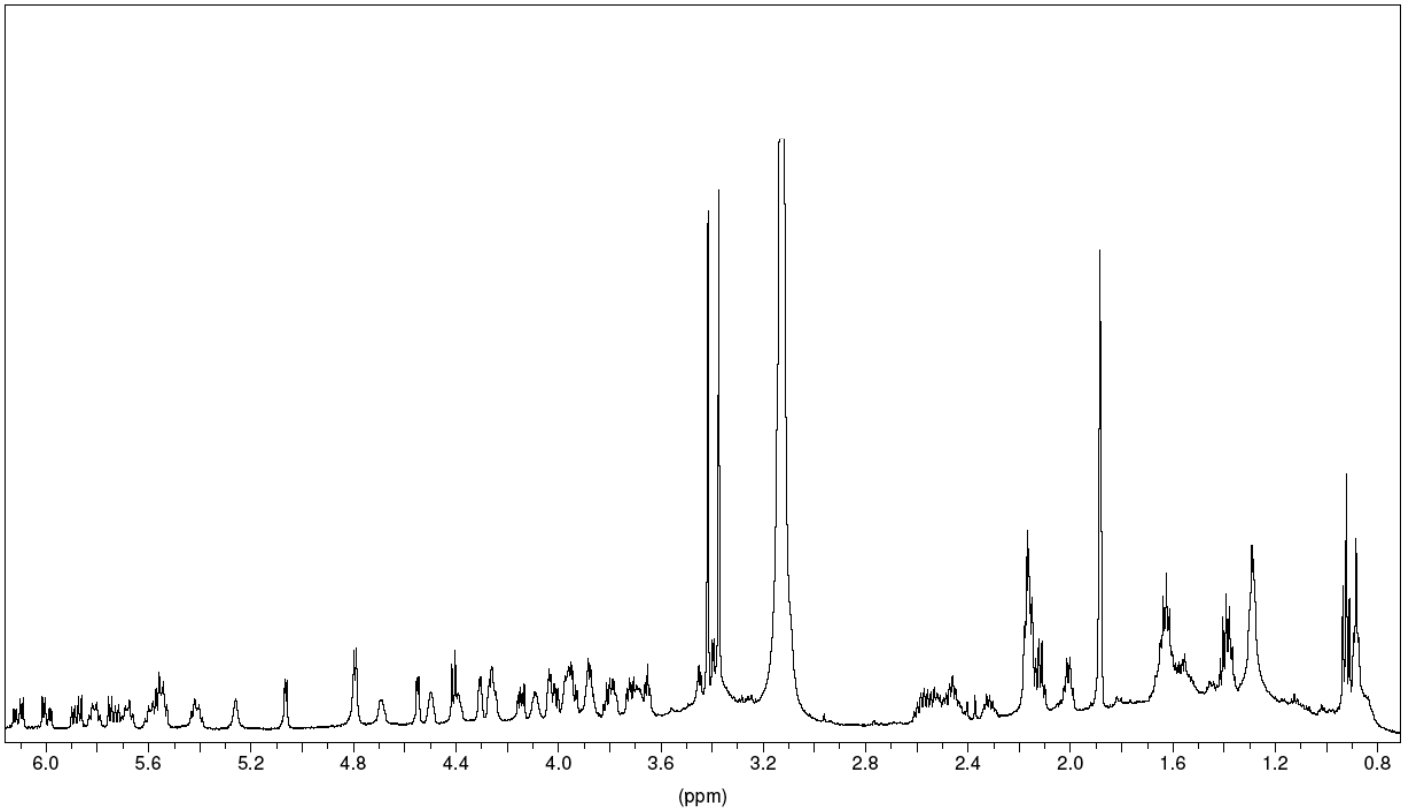
Table 1. ^{1}H and ^{13}C NMR Data for Nigricanoside A & B Dimethyl Esters (**3** & **4**) recorded in 540 µL of 25:2 C_6D_6/DMSO-d_6

	1[a]		**2**[a]	
Atom #	^{1}H (δ)	^{13}C (δ)	^{1}H (δ)	^{13}C (δ)
1		173.5		173.4
2	2.17 2.17	34.16	2.17 t *J*=7.4 Hz 2.17 t *J*=7.4 Hz	34.16
3	1.62 1.62	25.36	1.62 1.62	25.37
4	1.45 m 1.53 m	25.56	1.44 m 1.54	25.56
5	1.56 m 1.65	37.84	1.57 1.65	37.81
6	4.25	71.46	4.26	71.49
7	6.00 dd *J*=15.5,5.5 Hz	136.29	6.01 dd *J*=15.7,5.2 Hz	136.29
8	6.11 dd *J*=15.5,6.4 Hz	129.34	6.12 dd *J*=15.7,6.6 Hz	129.34
9	4.50 m	73.17	4.51	73.15
10	3.78 m	81.74	3.80	81.70
11	2.45 m 2.58 m	29.45	2.46 m 2.59 m	29.44
12	5.68 m	127.52	5.69 m	127.55
13	5.55 m	131.32[1]	5.56	131.55
14	2.12 ddd *J*=7.4,7.4,7.4 Hz 2.12 ddd *J*=7.4,7.4,7.4 Hz	29.87	2.12 ddd *J*=7.4,7.4,7.4 Hz 2.12 ddd *J*=7.4,7.4,7.4 Hz	29.88
15	1.40 qdd *J*=7.4,7.4,7.4 Hz 1.40 qdd *J*=7.4,7.4,7.4 Hz	23.16	1.40 qdd *J*=7.4,7.4,7.4 Hz 1.40 qdd *J*=7.4,7.4,7.4 Hz	23.18
16	0.92 t *J*=7.4 Hz	14.11	0.93 t *J*=7.4 Hz	14.12
1-O<u>Me</u>	3.41 s	51.10	3.41 s	51.10
6-O<u>H</u>	4.55 d *J*=4.5 Hz		4.49 d *J*=4.1 Hz	
9-O<u>H</u>	5.06 d *J*=4.6 Hz		5.04 t *J*=4.8 Hz	
1’		173.6		173.5
2’	2.16 2.16	33.38	2.16 t *J*=6.5 Hz 2.16 t *J*=6.5 Hz	33.38
3’	1.62 1.62	25.05	1.62 1.62	25.05
4’	2.01 ddd *J*=7.4,7.4,7.4 Hz	26.98	2.00 ddd *J*=7.4,7.4,7.4 Hz	26.99
5’	5.41 m	130.52	5.41	130.58
6’	5.59 m	126.99	5.59 m	126.97
7’	2.32 m 2.48	34.16	2.32 m 2.47	34.16
8’	3.80	80.84	3.81	80.83
9’	5.74 dd *J*=15.7,7.6 Hz	134.70	5.74 dd *J*=15.7,7.6 Hz	134.78
10’	5.88 dd *J*=15.7,7.5 Hz	131.34[1]	5.88 dd *J*=15.7,7.4 Hz	131.35

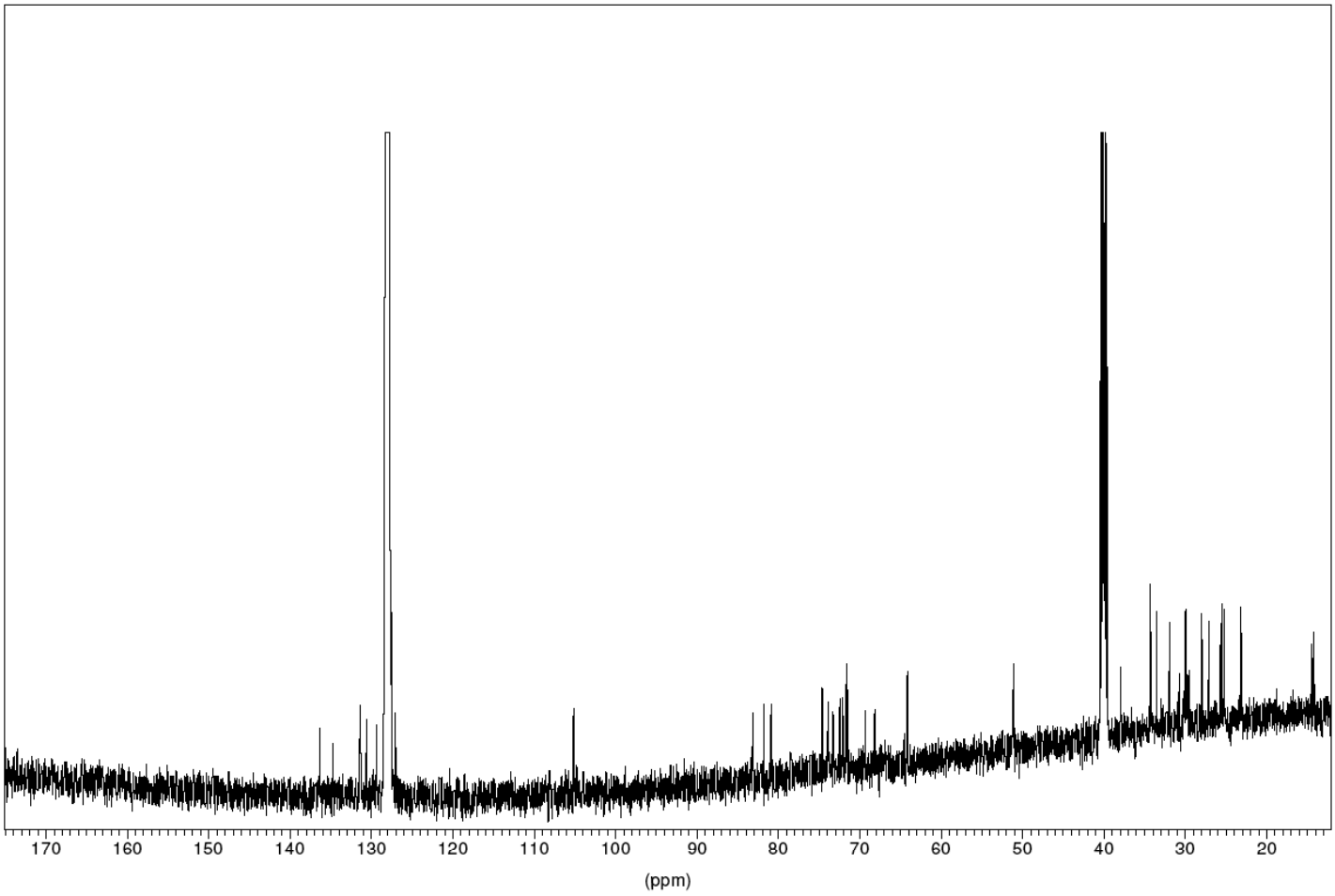
11’	4.26	83.08	4.27	83.02
12’	3.96	73.84	3.97	73.76
13’	2.53 2.53	30.66	2.53 m 2.58 m	30.63
14’	5.81 m	127.55	5.82 m	128.04
15’	5.55 m	131.27	5.55	129.52
16’	2.15 2.15	27.85	2.95 t *J*=7.0 Hz 2.95 t *J*=7.0 Hz	26.22
17’	1.29 1.38	29.77	5.46 m	127.90
18’	1.29 1.29	31.84	5.43	132.00
19’	1.29 1.29	22.95	2.07 qd *J*=7.3,7.3 Hz 2.07 qd *J*=7.3,7.3 Hz	20.93
20’	0.88 bt *J*=7.0 Hz	14.31	0.94 t *J*=7.3 Hz	14.29
1’-O<u>Me</u>	3.37 s	51.0	3.37 s	51.00
12’-O<u>H</u>	4.79 d *J*=5.7 Hz		4.80 d *J*=5.8 Hz	
1”	4.41 d *J*=7.7 Hz	105.11	4.41 d *J*=7.7 Hz	105.10
2”	3.95	71.98	3.97	72.04
3”	3.69 m	74.50[2]	3.69 m	74.51
4”	4.03 m	69.22	4.04 m	69.25
5”	3.65 bt *J*=6.1 Hz	74.51[2]	3.65 bt *J*=6.0 Hz	74.51
6”	3.72 dd *J*=9.6,6.1 Hz 4.01 dd *J*=9.6,6.1 Hz	68.06	3.73 dd *J*=9.3,6.0 Hz 4.02 dd *J*=9.3,6.0 Hz	68.03
2”-O<u>H</u>	5.26 bs		5.24 bs	
3”-O<u>H</u>	4.68 bs		4.63 bs	
4”-O<u>H</u>	4.30 d *J*=3.8 Hz		4.27	
1”’	3.94 dd *J*=10.4,4.0Hz 4.14 dd *J*=10.4,5.6 Hz	72.40	3.94 dd *J*=10.6,3.8 Hz 4.15 dd *J*=10.6,5.6 Hz	72.41
2”’	4.09 m	71.58	4.09 m	71.58
3”’	3.88 m 3.88 m	64.07	3.88 m 3.88 m	64.09
2”’-O<u>H</u>	4.79 d *J*=5.7 Hz		4.77 d *J*=5.5 Hz	
3”’-O<u>H</u>	4.39 m		4.33 t *J*=5.9 Hz	

[1,2]Assignments within a column are interchangeable

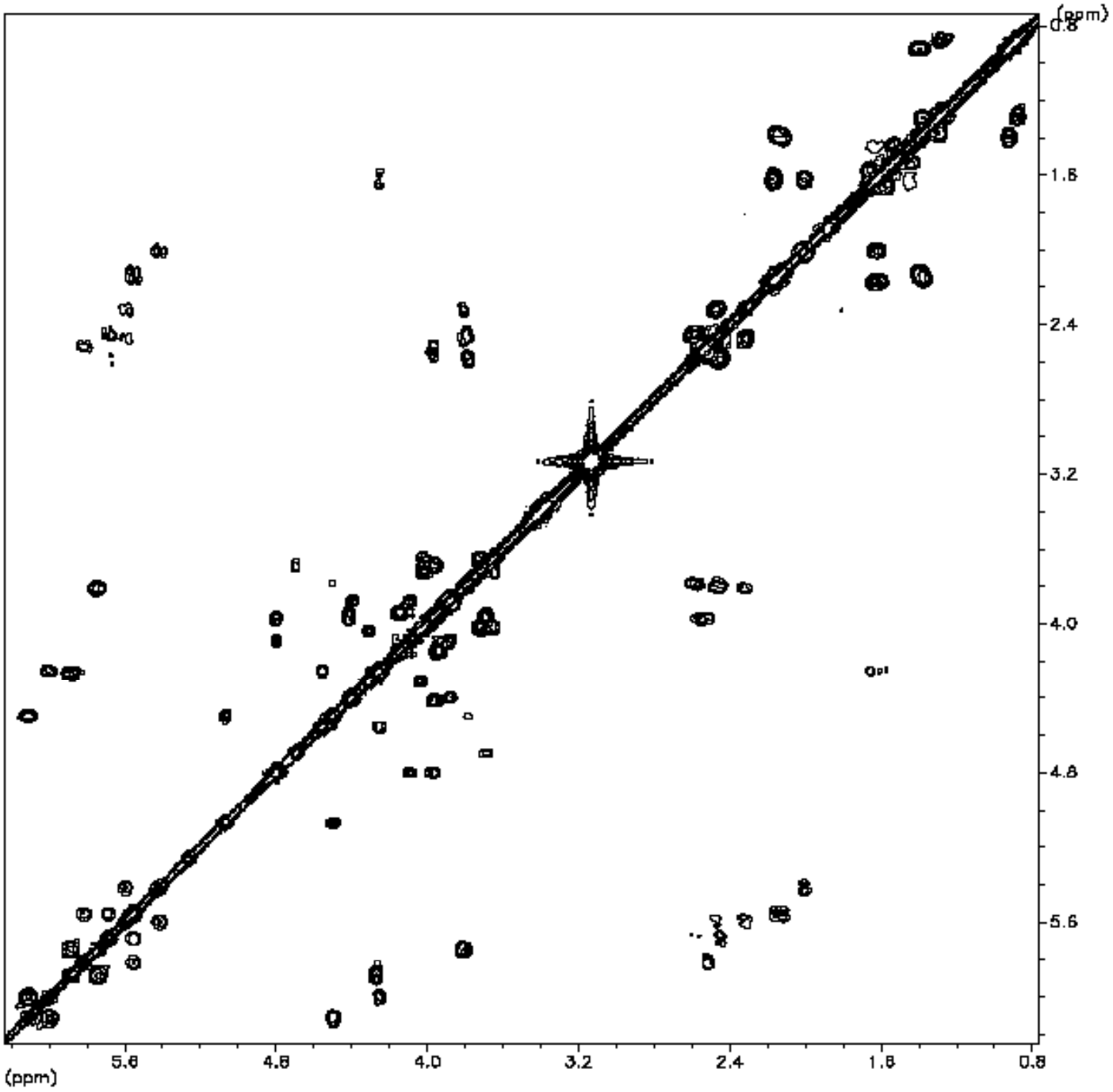
^{1}H NMR Spectrum of Nigricanoside A Dimethyl Ester (**3**) recorded at 600 MHz in 25:2 C_6D_6/DMSO-d_6



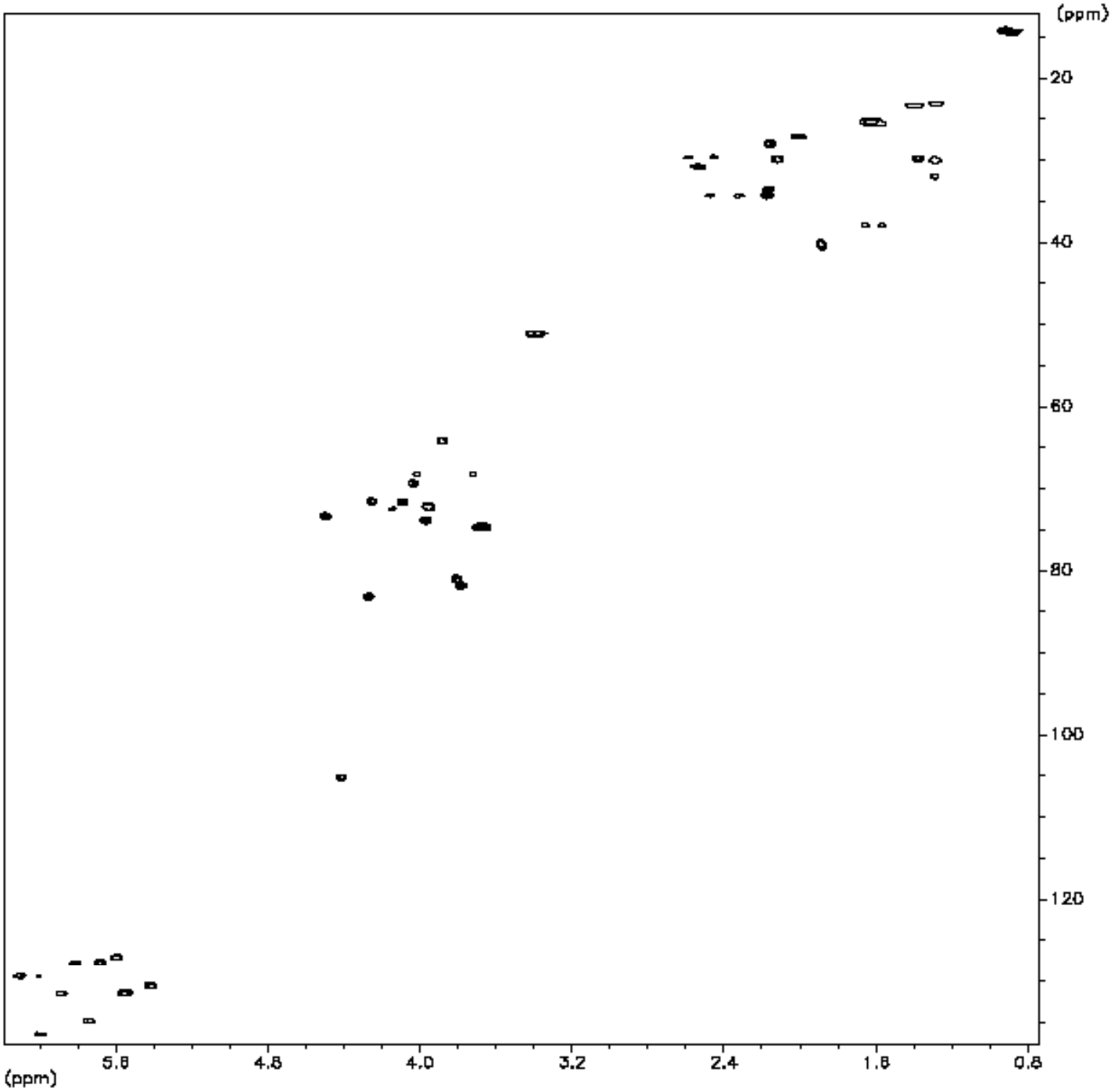
^{13}C NMR Spectrum of Nigricanoside A Dimethyl Ester (**3**) recorded at 150 MHz in 25:2 C_6D_6/DMSO-d_6



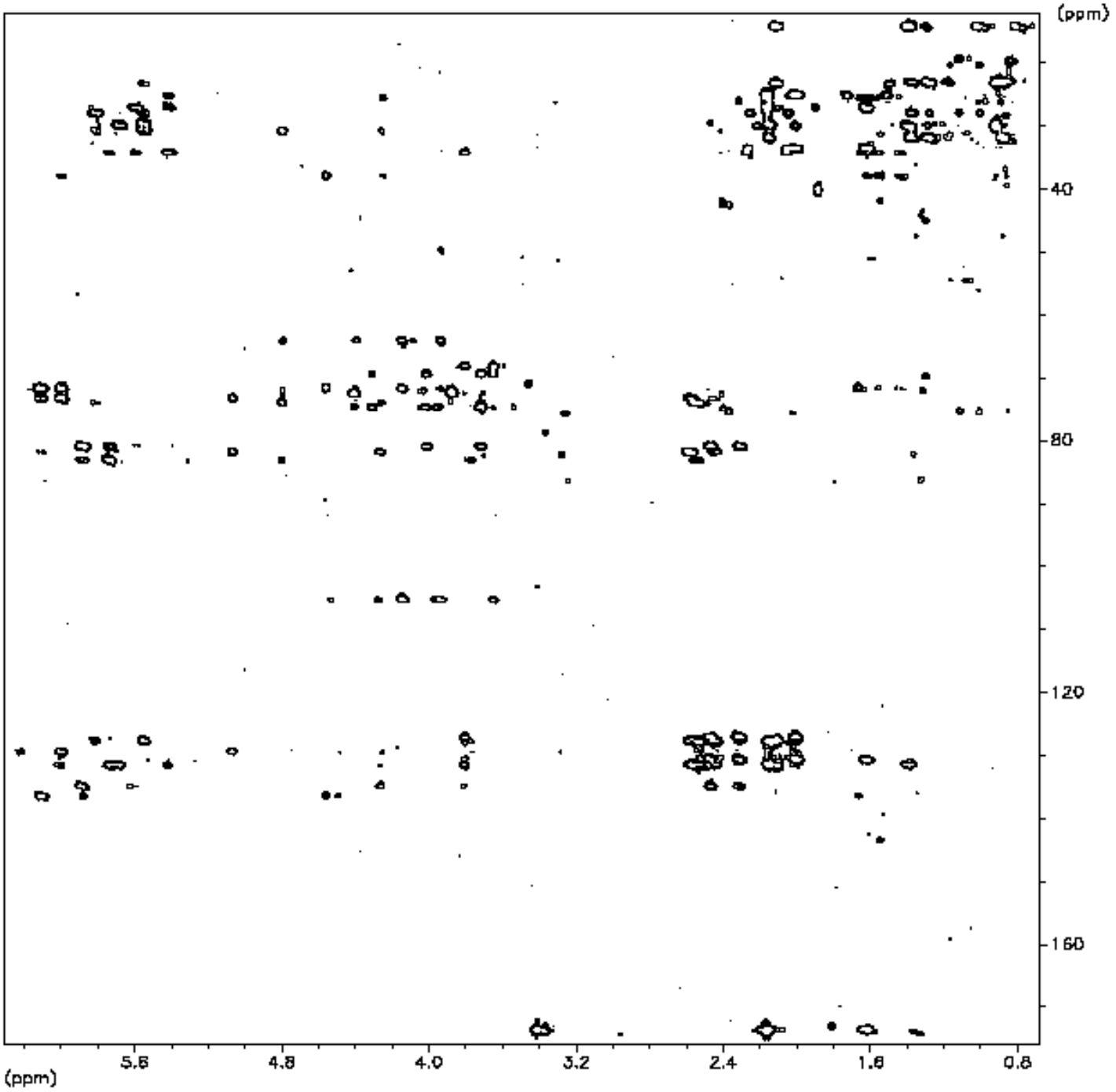
gradCOSY60 NMR Spectrum of Nigricanoside A Dimethyl Ester (**3**) recorded at 600 MHz in 25:2 C_6D_6/DMSO-d_6



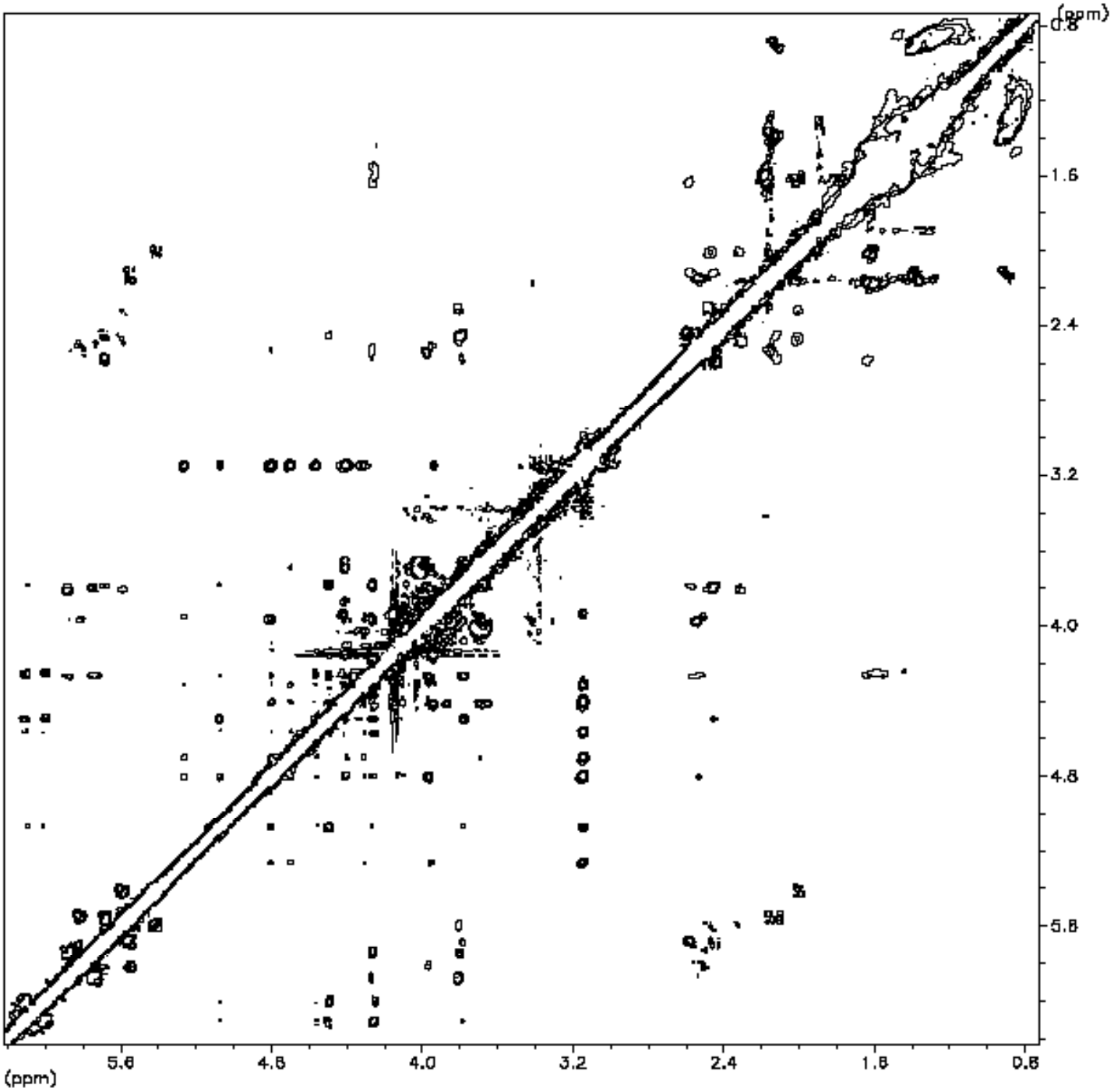
gradHSQC NMR Spectrum of Nigricanoside A Dimethyl Ester (**3**) recorded at 600 MHz in 25:2 C_6D_6/DMSO-d_6



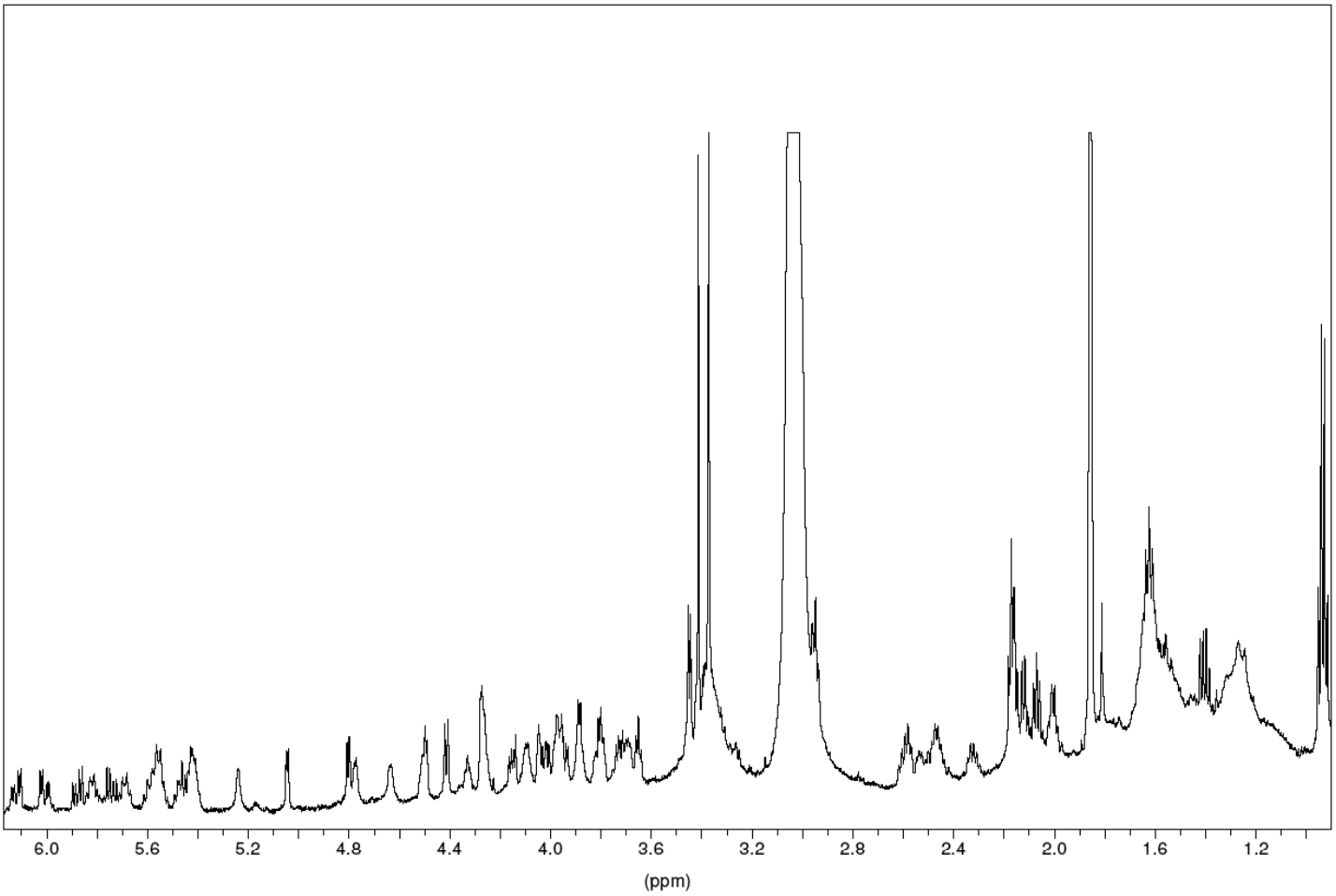
gradHMBC NMR Spectrum of Nigricanoside A Dimethyl Ester (**3**) recorded at 600 MHz in 25:2 C_6D_6/DMSO-d_6



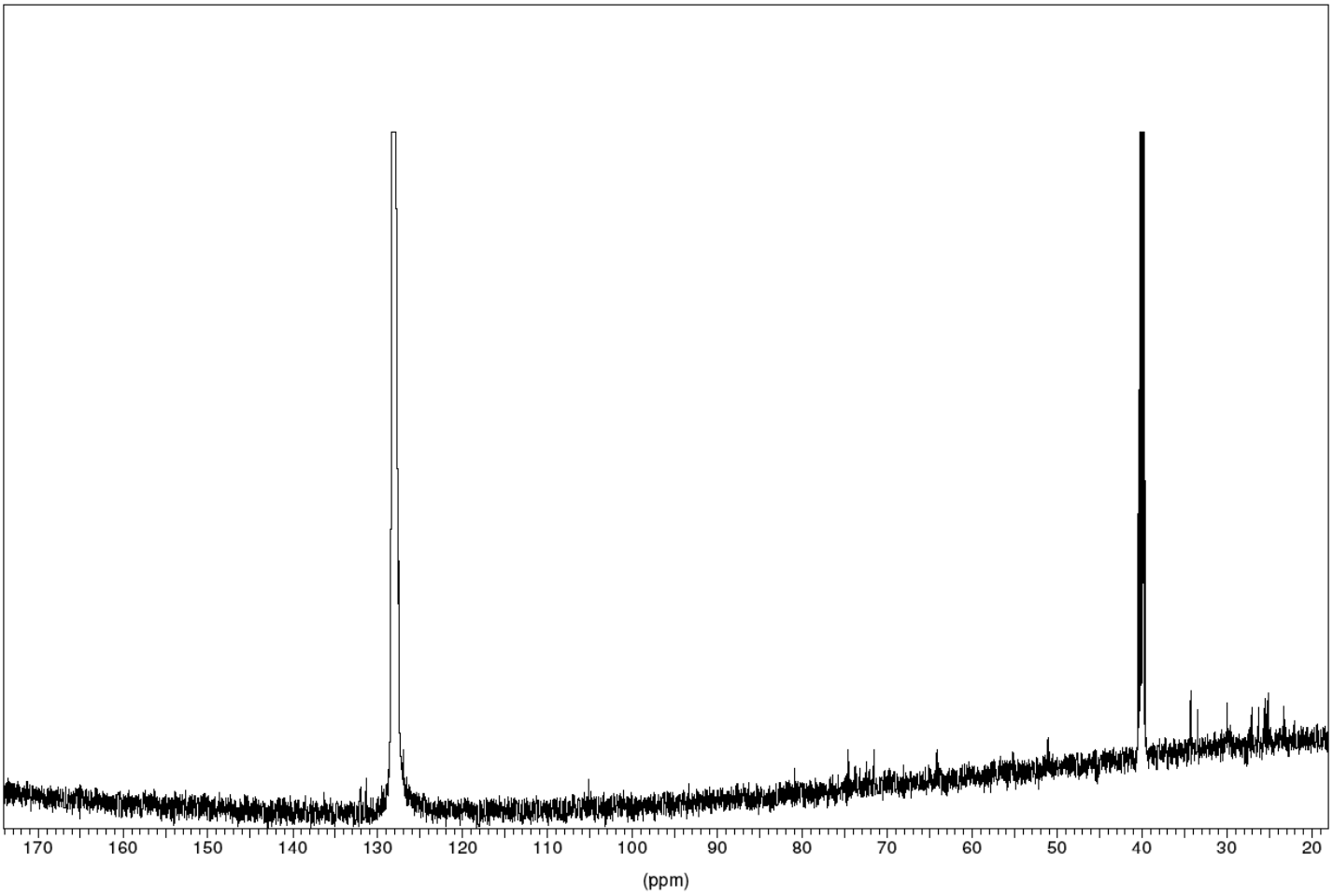
ROESY NMR Spectrum of Nigricanoside A Dimethyl Ester (**3**) recorded at 600 MHz in 25:2 C_6D_6/DMSO-d_6



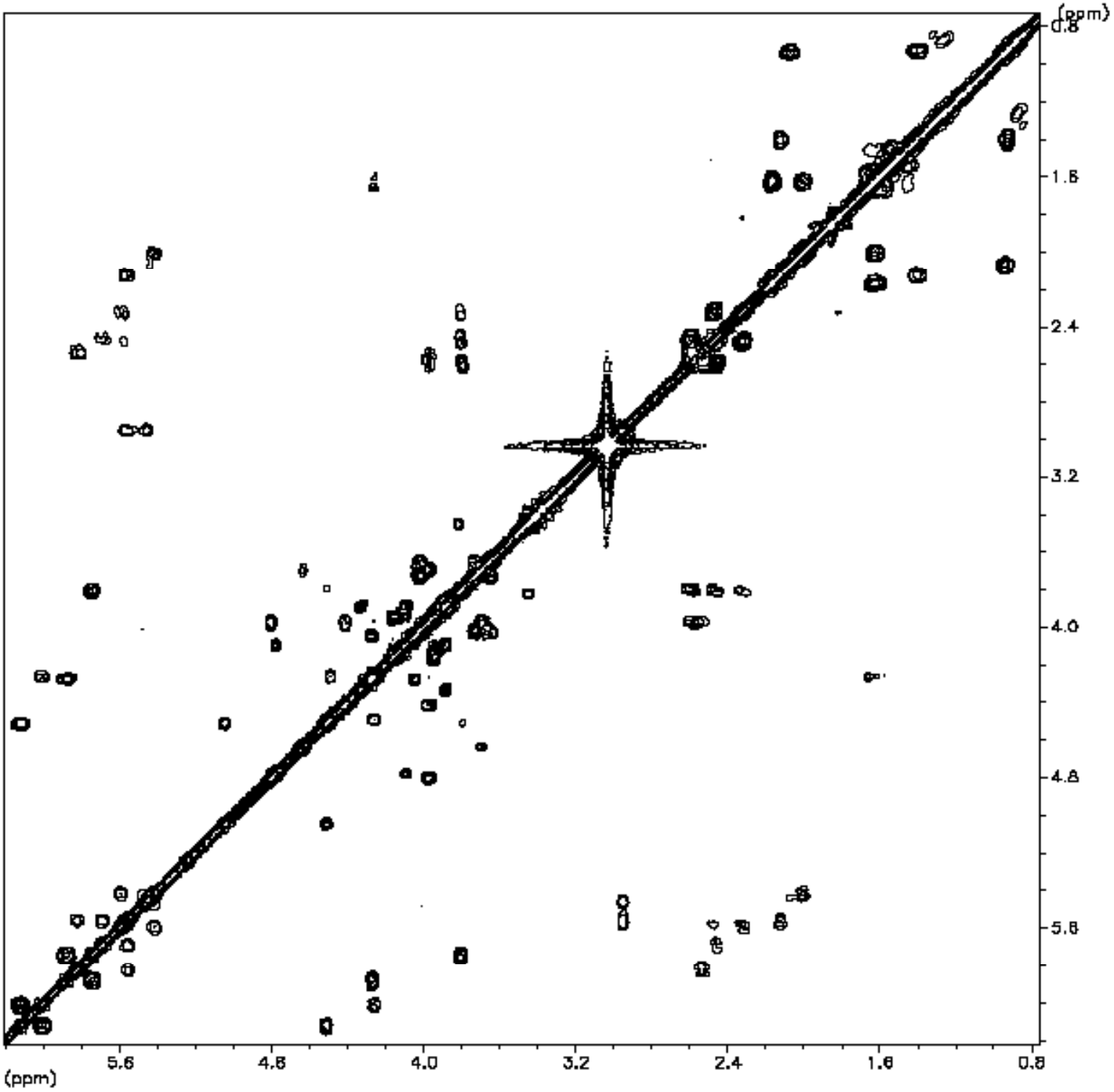
^{1}H NMR Spectrum of Nigricanoside B Dimethyl Ester (**4**) recorded at 600 MHz in 25:2 C_6D_6/DMSO-d_6



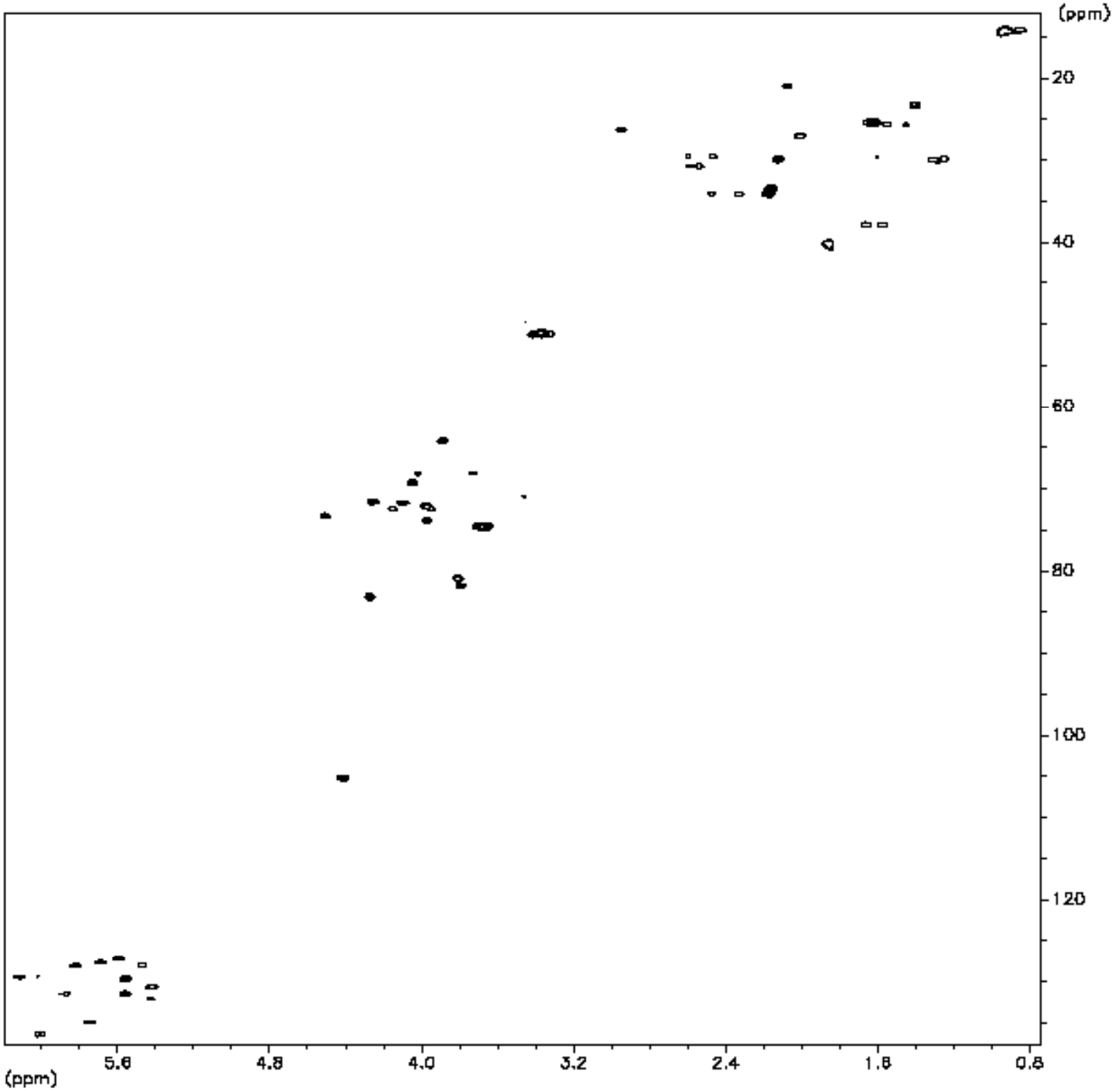
^{13}C NMR Spectrum of Nigricanoside B Dimethyl Ester (**4**) recorded at 150 MHz in 25:2 C_6D_6/DMSO-d_6



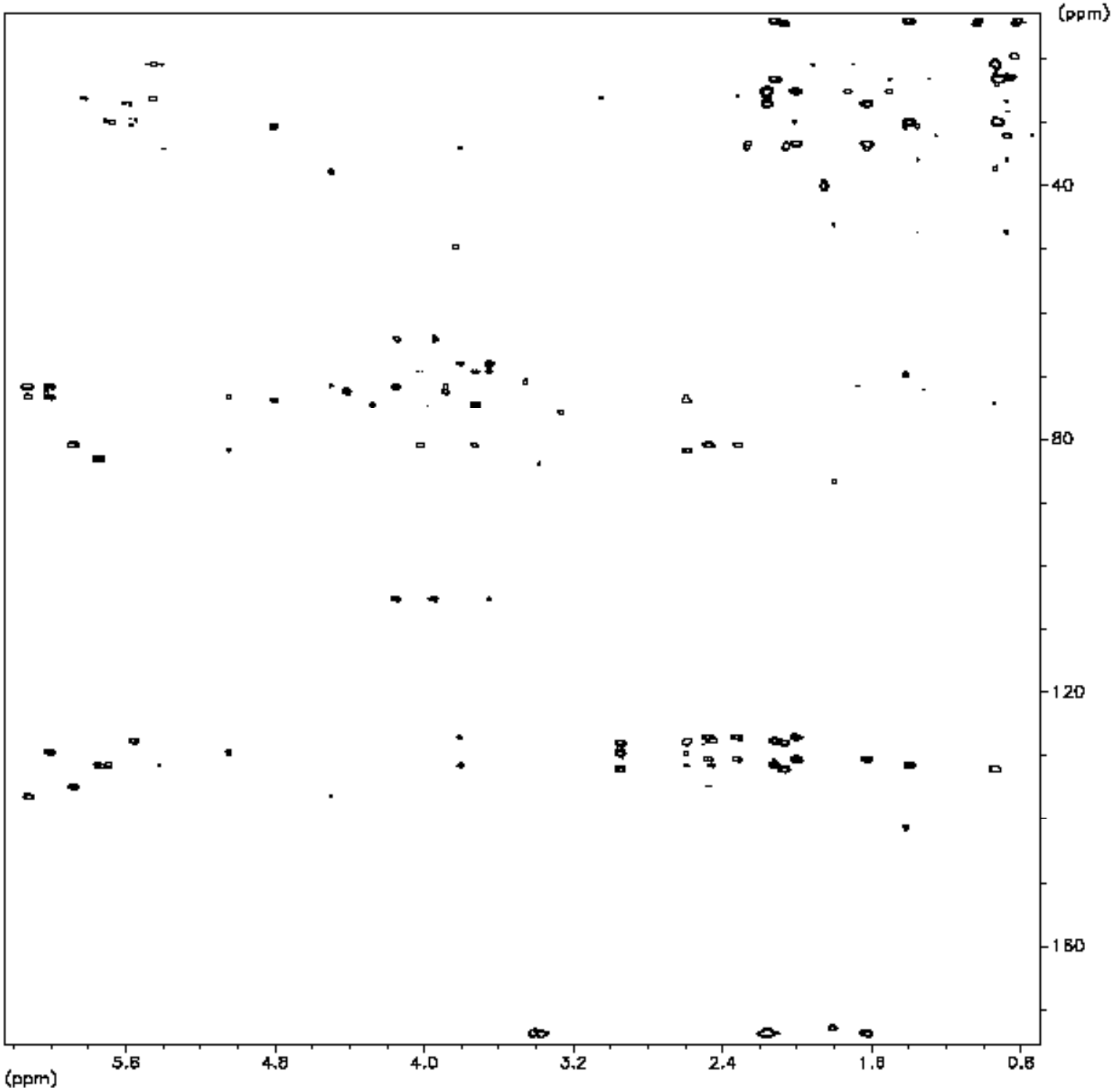
gradCOSY60 NMR Spectrum of Nigricanoside B Dimethyl Ester (**4**) recorded at 600 MHz in 25:2 C_6D_6/DMSO-d_6



gradHSQC NMR Spectrum of Nigricanoside B Dimethyl Ester (**4**) recorded at 600 MHz in 25:2 C_6D_6/DMSO-d_6



gradHMBC NMR Spectrum of Nigricanoside B Dimethyl Ester (**4**) recorded at 600 MHz in 25:2 C_6D_6/DMSO-d_6



ROESY NMR Spectrum of Nigricanoside B Dimethyl Ester (**4**) recorded at 600 MHz in 25:2 C_6D_6/DMSO-d_6