Supporting information to:

Cytotoxic Properties of Root Extract and Fruit Juice of *Trichosanthes cucumerina*

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Chromatographic and Spectroscopic Equipment and Isolation Methods

Spectroscopic equipment

1D and 2D 400-MHz ^{1}H- and 100.6-MHz ^{13}C-NMR spectra were recorded on an Avance 400. Mass spectra were measured on a Hitachi M-60. Optical rotation measurements were recorded on an Atago Polax-L. Low-pressure liquid chromatography was performed with a WIZ peristaltic pump (Isco), a Foxy Jr. fraction collector (Isco), a UA-6 UV detector (Isco), and a 6-ported valve for sample injection.

High-pressure liquid chromatography

HPLC was performed on a Shimadzu Class VP instrument with an LC 10AD VP pump, an SCL-10A VP system controller, an SPD-10AV VP UV-VIS detector, and an SIL-10AD VP auto injector. The following chromatographic conditions were used: column: 5 μm HYPERSIL BDS C18; solvent system: cucurbitacin B: methanol–water (65:35), bryonolic acid: methanol–water (95:5); flow rate: 1 mL/min; detection: 210 nm; volume: 20 μL.

Plant materials

The plant roots and green mature fruits were collected in September 2000 from the Pichit Horticultural Research Center, Department of Agriculture, Ministry of Agriculture and Cooperatives, Pichit Province, Thailand. The roots were washed, sliced into pieces, dried in an oven (60 °C), and powdered. The fruits were washed and pressed. Plant identification and preservation of the voucher specimen (BKF No. 70279) were carried out by the Royal Forest Department, Ministry of Natural Resources and Environment, Bangkok, Thailand.

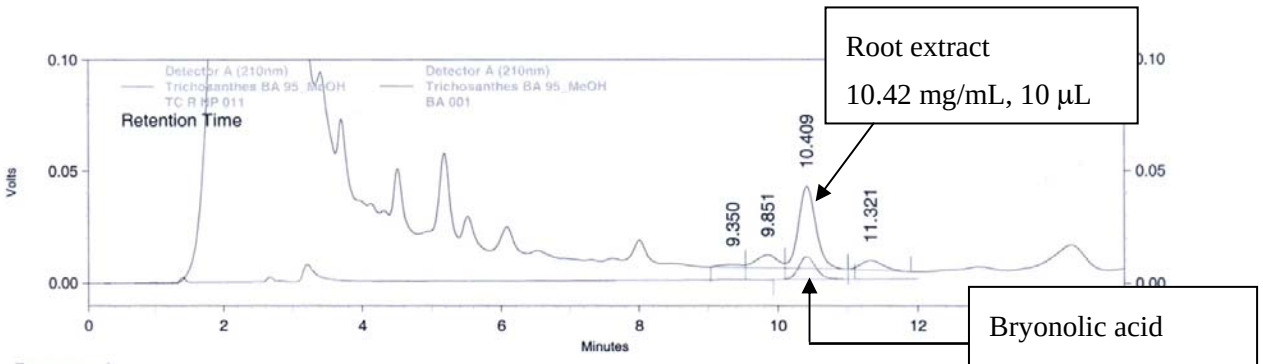
Plant extraction

Dried, ground root (2 kg) was successively extracted in a Soxhlet apparatus with 4 L petroleum ether (40–60 °C), 4 L dichloromethane, and 4 L ethanol. The dichloromethane dry extract possessed a drug extract ratio of 1:60. The fresh fruits (21 kg) were squeezed to yield 2 L juice, which was shaken with diethyl ether. The ether extract was concentrated.

Isolation of triterpenes

During the concentration of the dichloromethane root extract under reduced pressure, compound **1** (1.23 g) crystallized. The remaining dry extract (20 g) was transferred to column

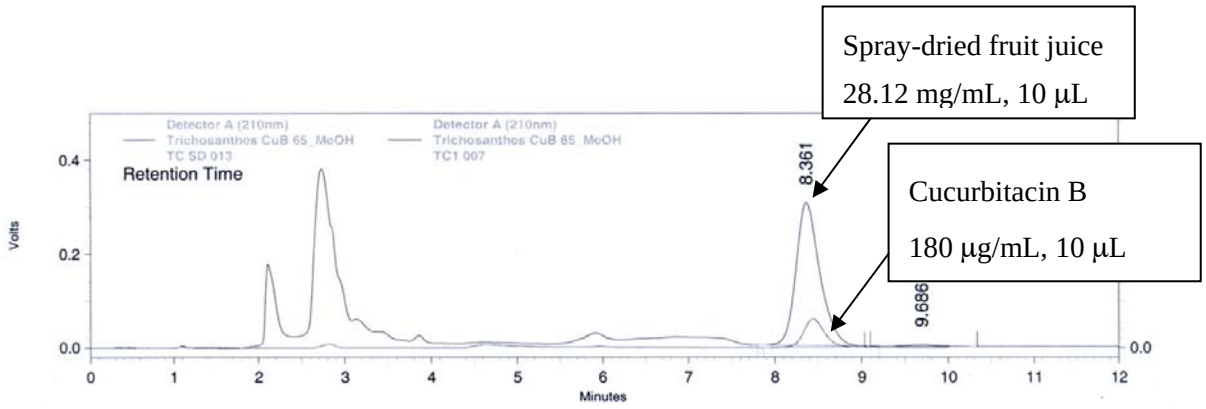
chromatography (500 g, silica gel). The column was eluted with dichloromethane and, gradiently, with dichloromethane-methanol (1% → 6%). The eluting solvent volume was 400 mL for each concentration. Retention volumes of 650–1150 mL (fraction V) and 1150–1250 mL (fraction VI) were collected and further chromatographed. Fraction V (972 mg) was transferred to column chromatography (100 g silica gel) and eluted with hexane:ethyl acetate (9:1). A retention volume of 330–340 mL (49 mg residue) was collected and recrystallized in methanol and compound **2** (6 mg) was obtained. Fraction VI (110 mg) was taken up in methanol and chromatographed on a Lobar column eluted with acetonitrile:water (55:45). Retention volumes of 58–72 mL and 75–84 mL were collected for compounds **3** (16 mg) and **4** (2 mg), respectively.



Detector A (210nm)

Pk #	Name	Retention Time	Area	Area Percent	Height	Asymmetry
1		9.350	26522	3.02	1186	0.00
2		9.851	124682	14.22	5894	0.00
3		10.409	634474	72.34	36174	1.14
4		11.321	91358	10.42	4291	1.24
Totals			877036	100.00	47544	

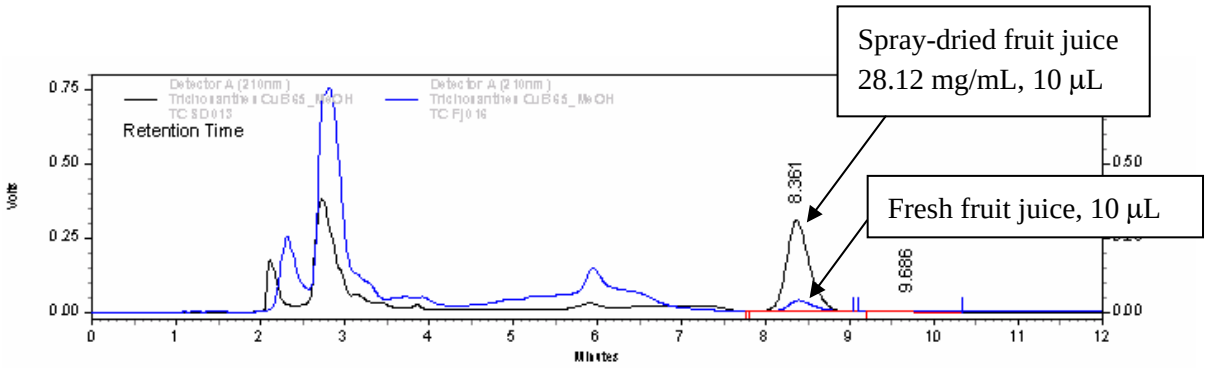
Fig. 1S HPLC chromatograms of the root extract and bryonolic acid (**1**).



Detector A (210nm)

Pk #	Name	Retention Time	Area	Area Percent	Height	Asymmetry
1		8.361	5791998	98.72	302443	1.26
2		9.686	74958	1.28	3207	1.09
Totals			5866956	100.00	305650	

Fig. 2S HPLC chromatograms of spray-dried fruit juice and cucurbitacin B (**3**).



Detector A (210nm)						
Pk#	Name	**Retention Time**	**Area**	Area Percent	Height	Asymmetry
1		8.361	5791998	98.72	302443	1.26
2		9.686	74958	1.28	3207	1.09
Totals						
			5866956	100.00	305650	

Fig. 3S HPLC chromatograms of spray-dried fruit juice and fresh fruit juice.

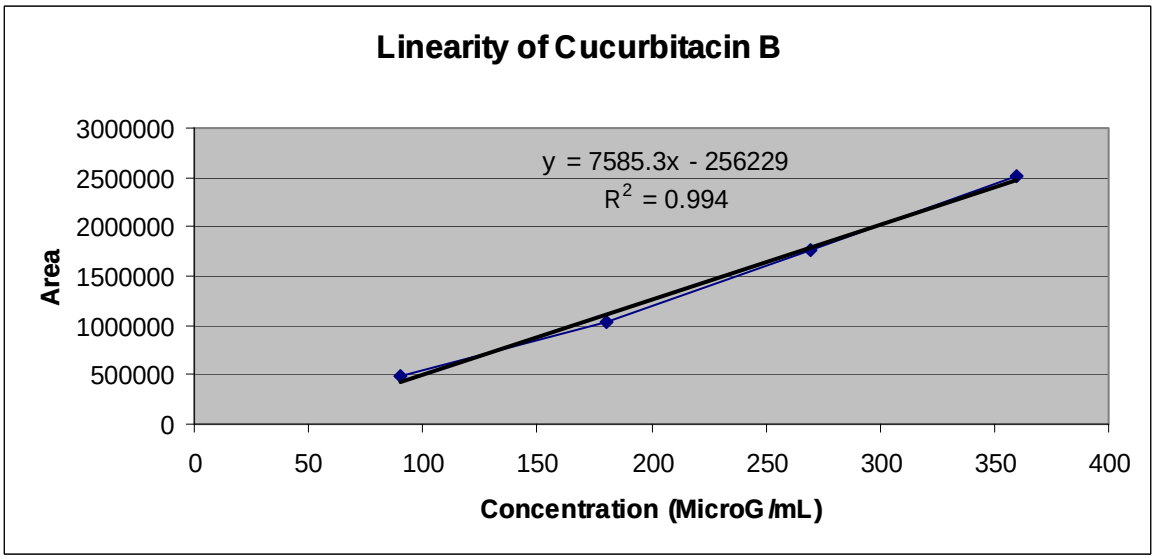


Fig. 4S Linearity graph of cucerbitacin B (**3**).

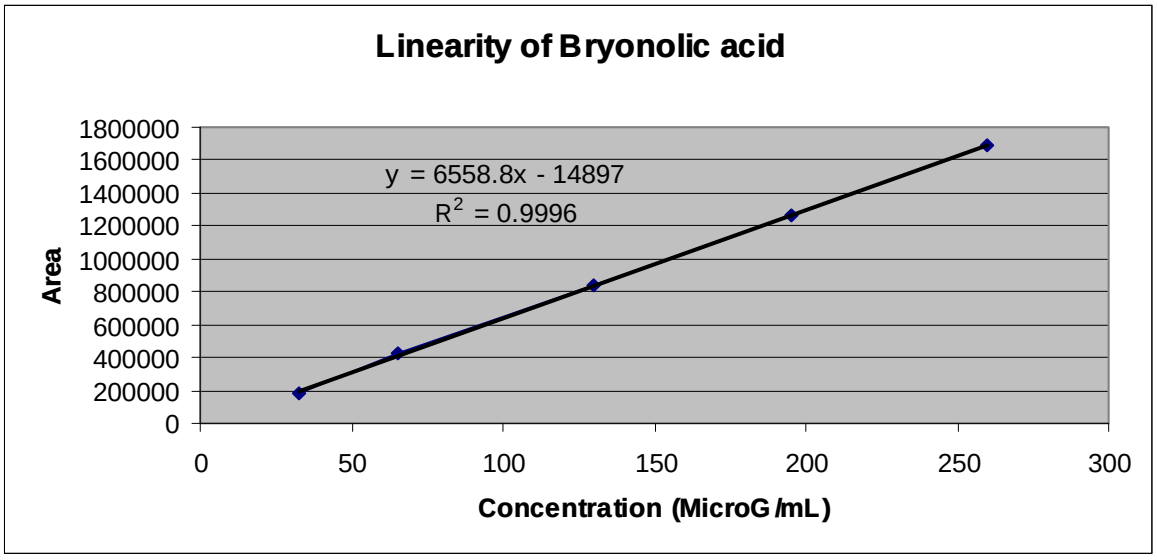
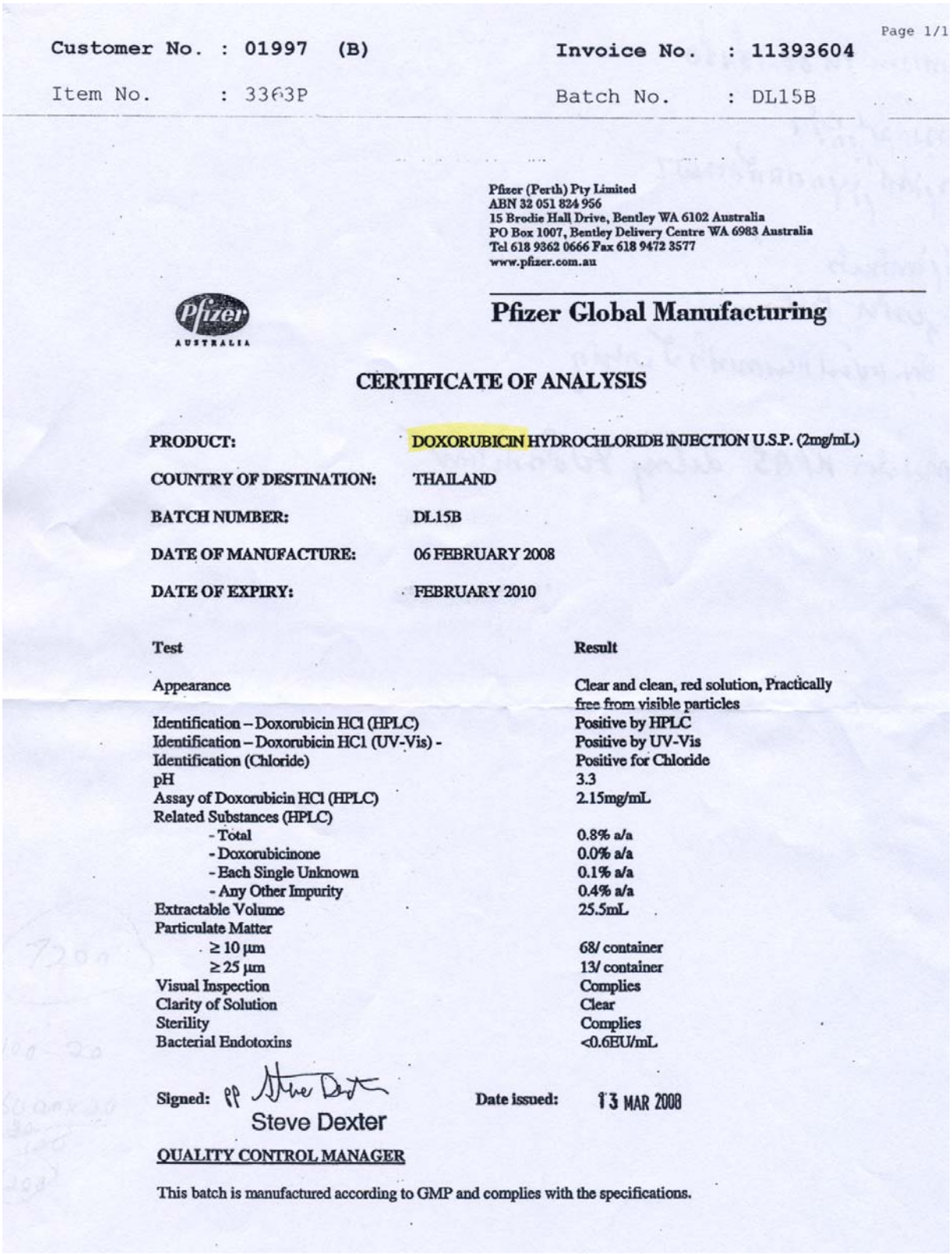


Fig. 5S Linearity graph of bryonolic acid (**1**).



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Customer No. : 01997 (B) Invoice No. : 11393604

Item No. : 3363P Batch No. : DL15B

Pfizer (Perth) Pty Limited
ABN 32 051 824 956
15 Brodie Hall Drive, Bentley WA 6102 Australia
PO Box 1007, Bentley Delivery Centre WA 6983 Australia
Tel 618 9362 0666 Fax 618 9472 3577
www.pfizer.com.au

Pfizer AUSTRALIA

Pfizer Global Manufacturing

CERTIFICATE OF ANALYSIS

PRODUCT:	DOXORUBICIN HYDROCHLORIDE INJECTION U.S.P. (2mg/mL)
COUNTRY OF DESTINATION:	THAILAND
BATCH NUMBER:	DL15B
DATE OF MANUFACTURE:	06 FEBRUARY 2008
DATE OF EXPIRY:	FEBRUARY 2010

Test	Result
Appearance	Clear and clean, red solution, Practically free from visible particles
Identification – Doxorubicin HCl (HPLC)	Positive by HPLC
Identification – Doxorubicin HCl (UV-Vis) -	Positive by UV-Vis
Identification (Chloride)	Positive for Chloride
pH	3.3
Assay of Doxorubicin HCl (HPLC)	2.15mg/mL
Related Substances (HPLC)	
- Total	0.8% a/a
- Doxorubicinone	0.0% a/a
- Each Single Unknown	0.1% a/a
- Any Other Impurity	0.4% a/a
Extractable Volume	25.5mL
Particulate Matter	
≥ 10 µm	68/ container
≥ 25 µm	13/ container
Visual Inspection	Complies
Clarity of Solution	Clear
Sterility	Complies
Bacterial Endotoxins	<0.6EU/mL

Signed: pp Steve Dexter Date issued: 13 MAR 2008

Steve Dexter

QUALITY CONTROL MANAGER

This batch is manufactured according to GMP and complies with the specifications.

Fig. 6S Document showing the origin and purity of doxorubicin (positive control).

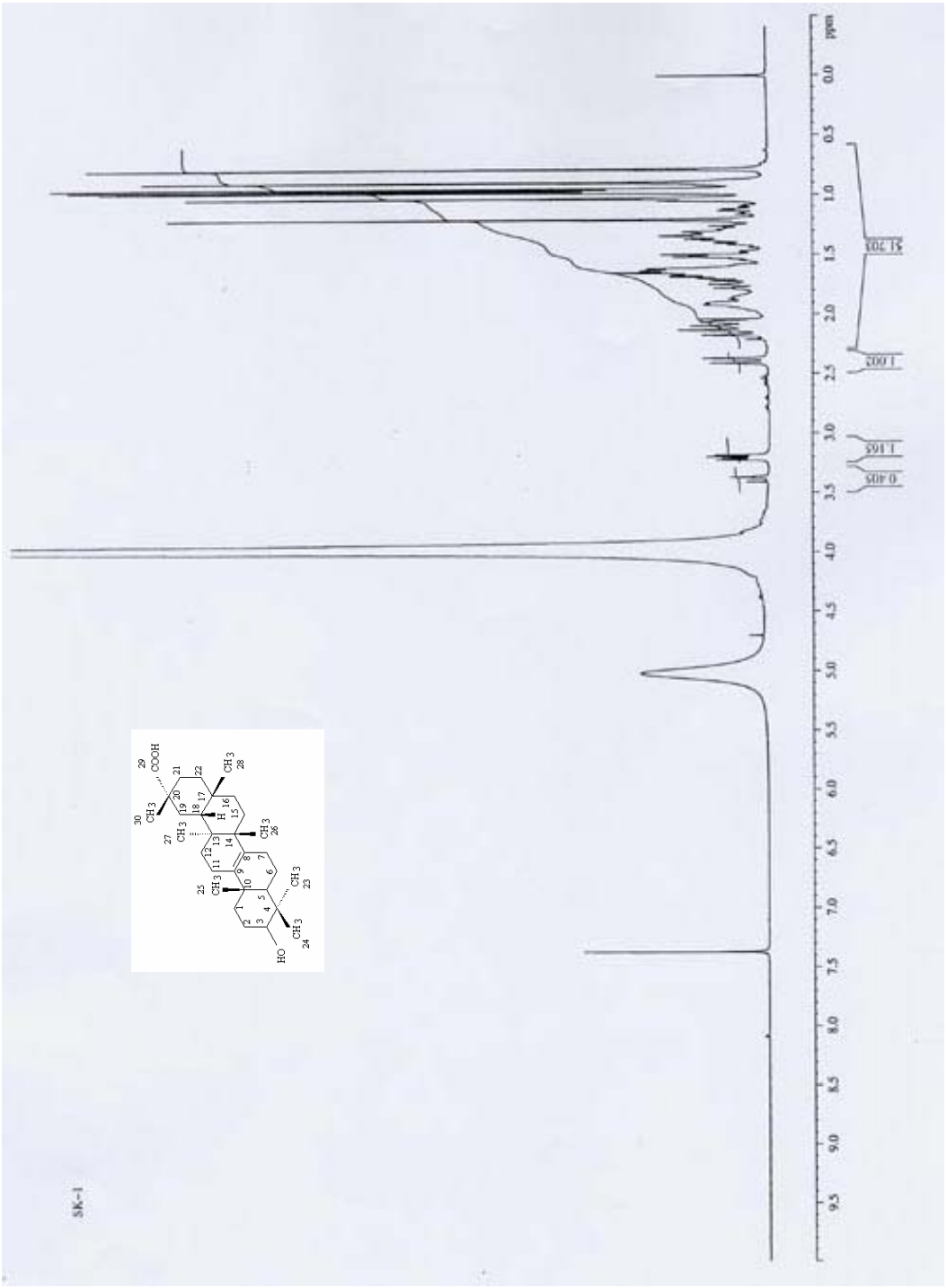

Fig. 7S 400-MHz ^{1}H-NMR spectrum of **1** in $CDCl_3$:CD_3OD.

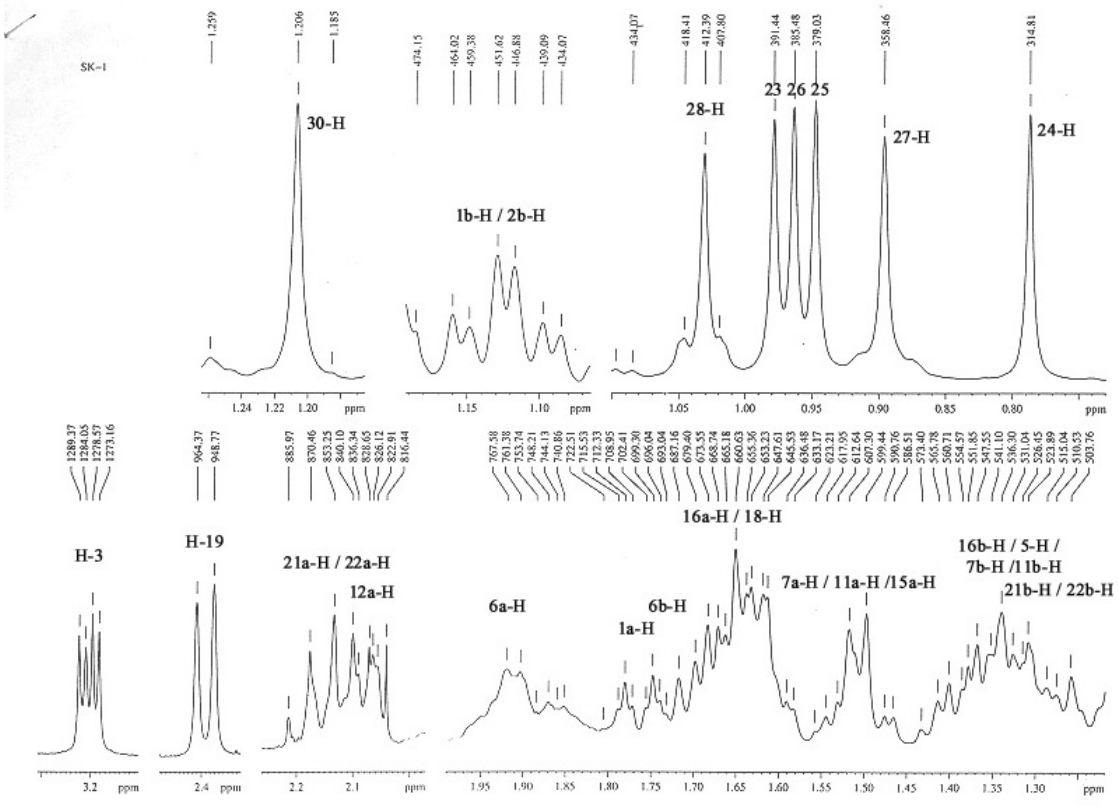

Fig. 8S Expanded 400-MHz ^{1}H-NMR spectrum of **1** in $CDCl_3$:CD_3OD (δ = 0–3.3).

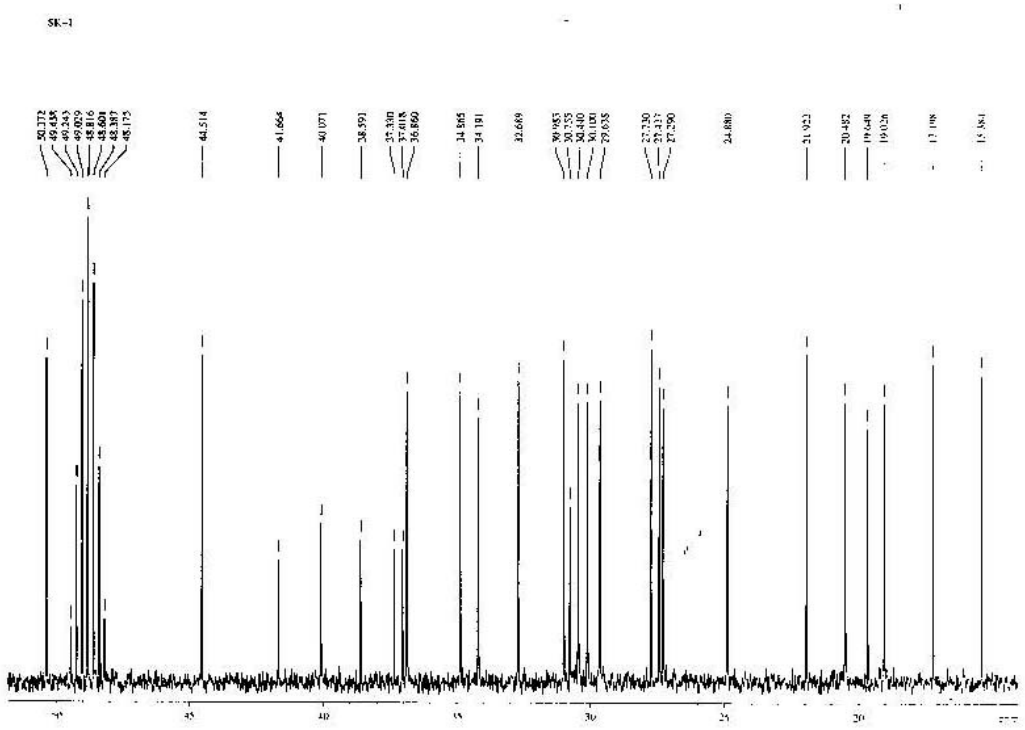

Fig. 9S $^{13}C\{^{1}H\}$ broadband decoupled spectrum of **1**.

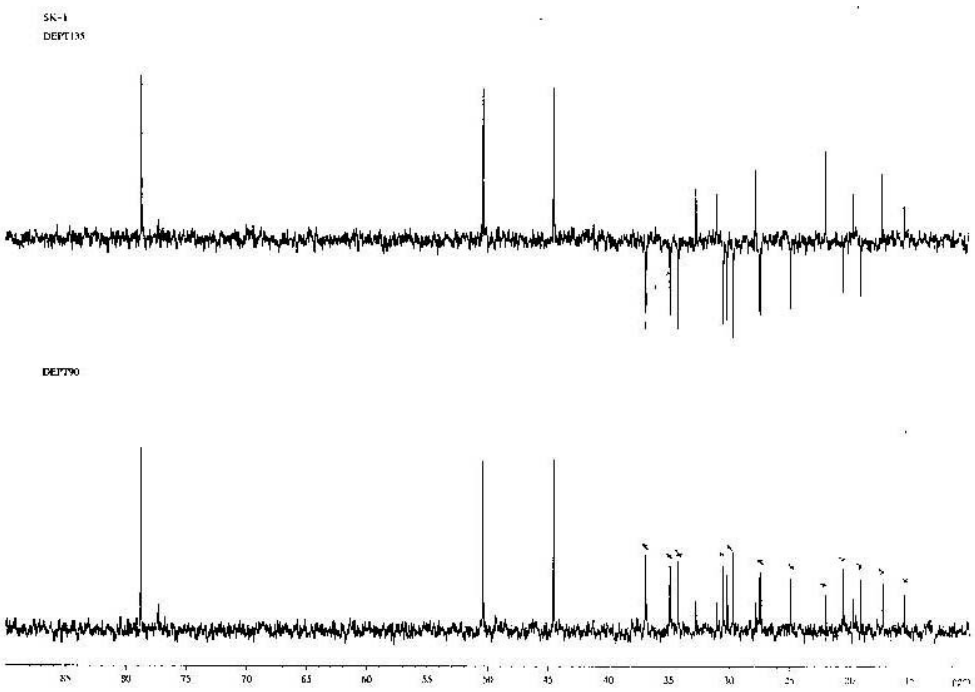

Fig. 10S DEPT spectrum of **1**.

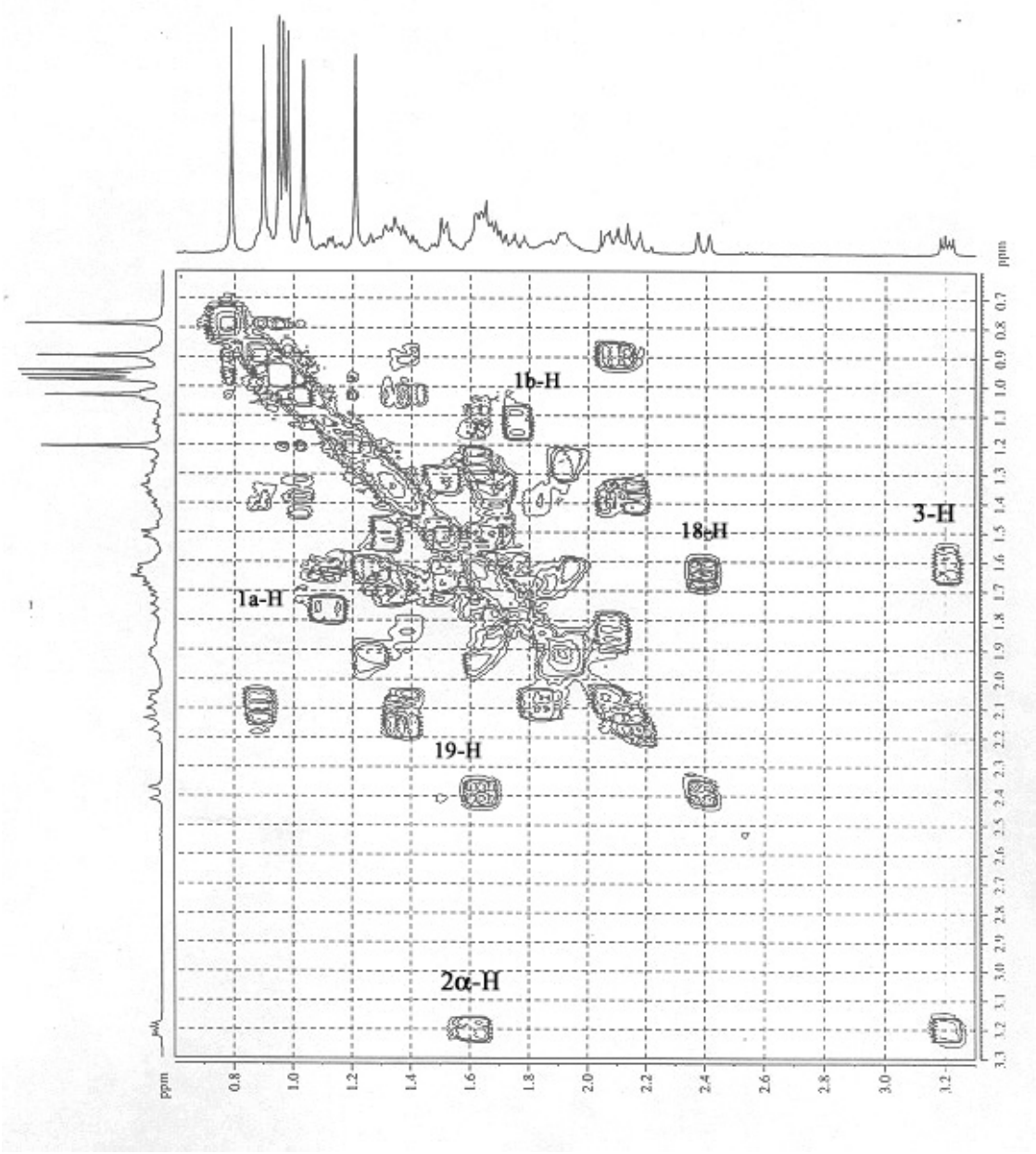

Fig. 11S H-H COSY spectrum of **1** in $CDCl_3$:CD_3OD.

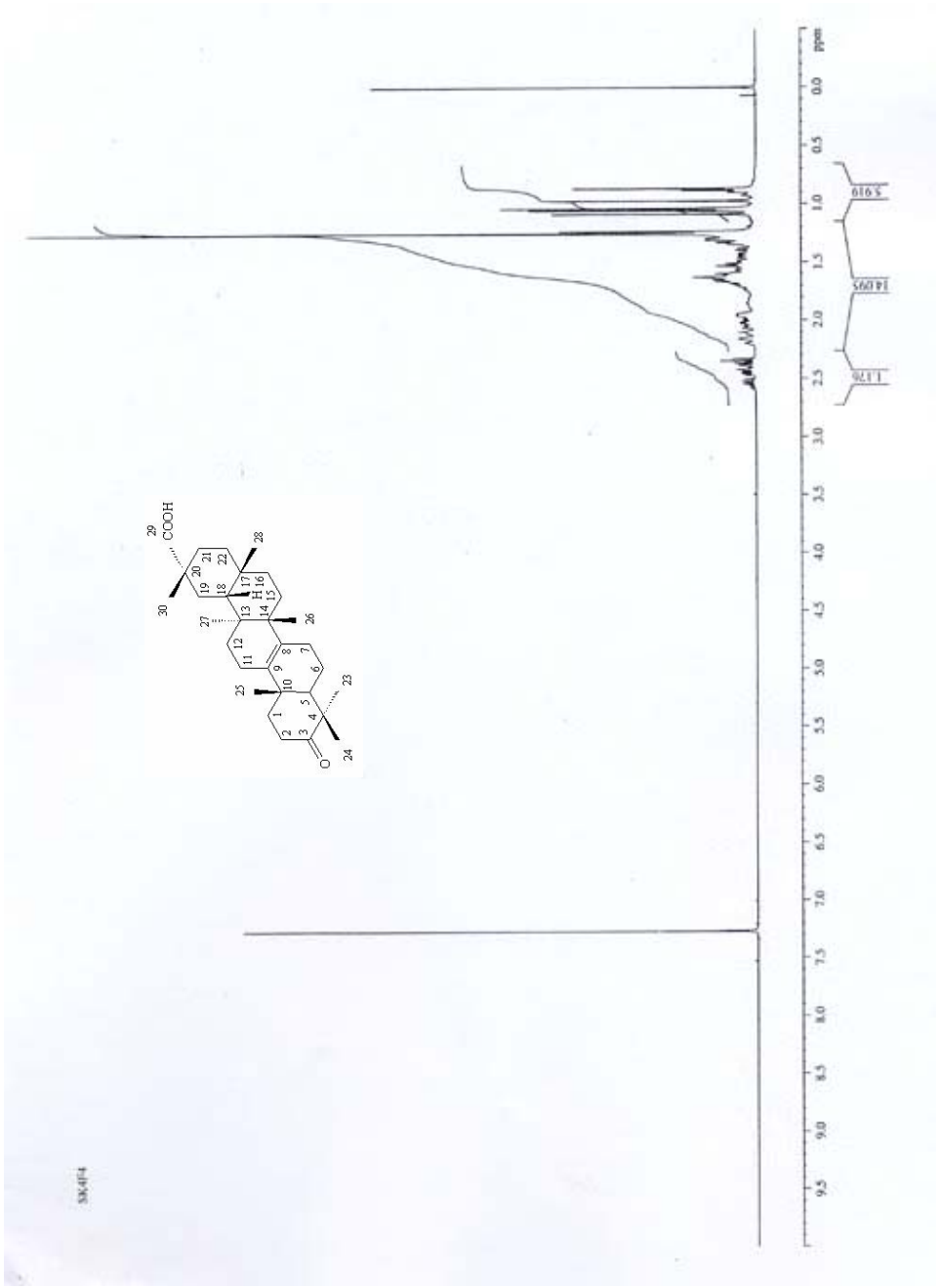

Fig. 12S 400-MHz ^{1}H-NMR spectrum of **2** in $CDCl_3$.

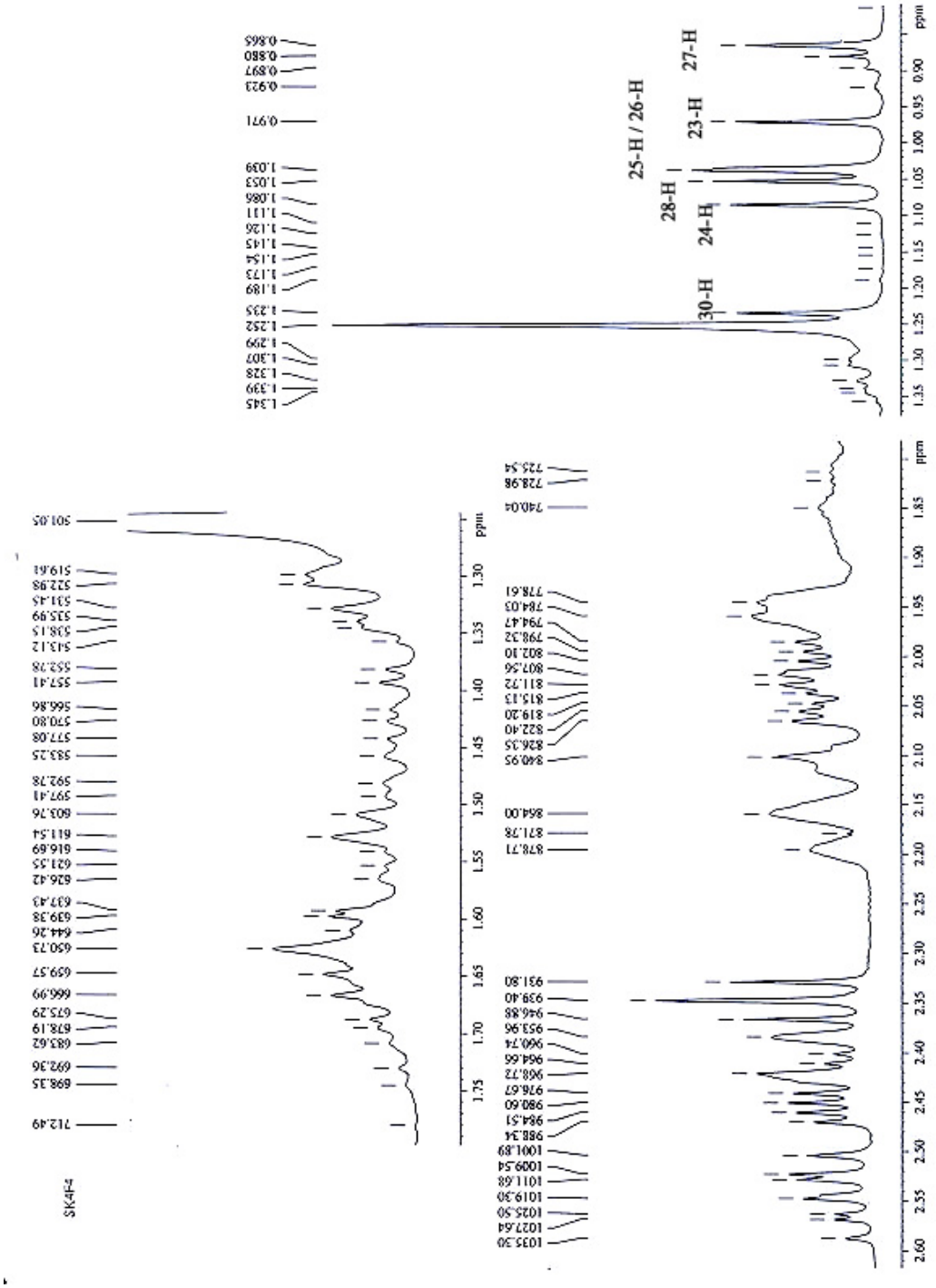

Fig. 13S Expanded 400-MHz ^{1}H-NMR spectrum of **2** in $CDCl_3$.

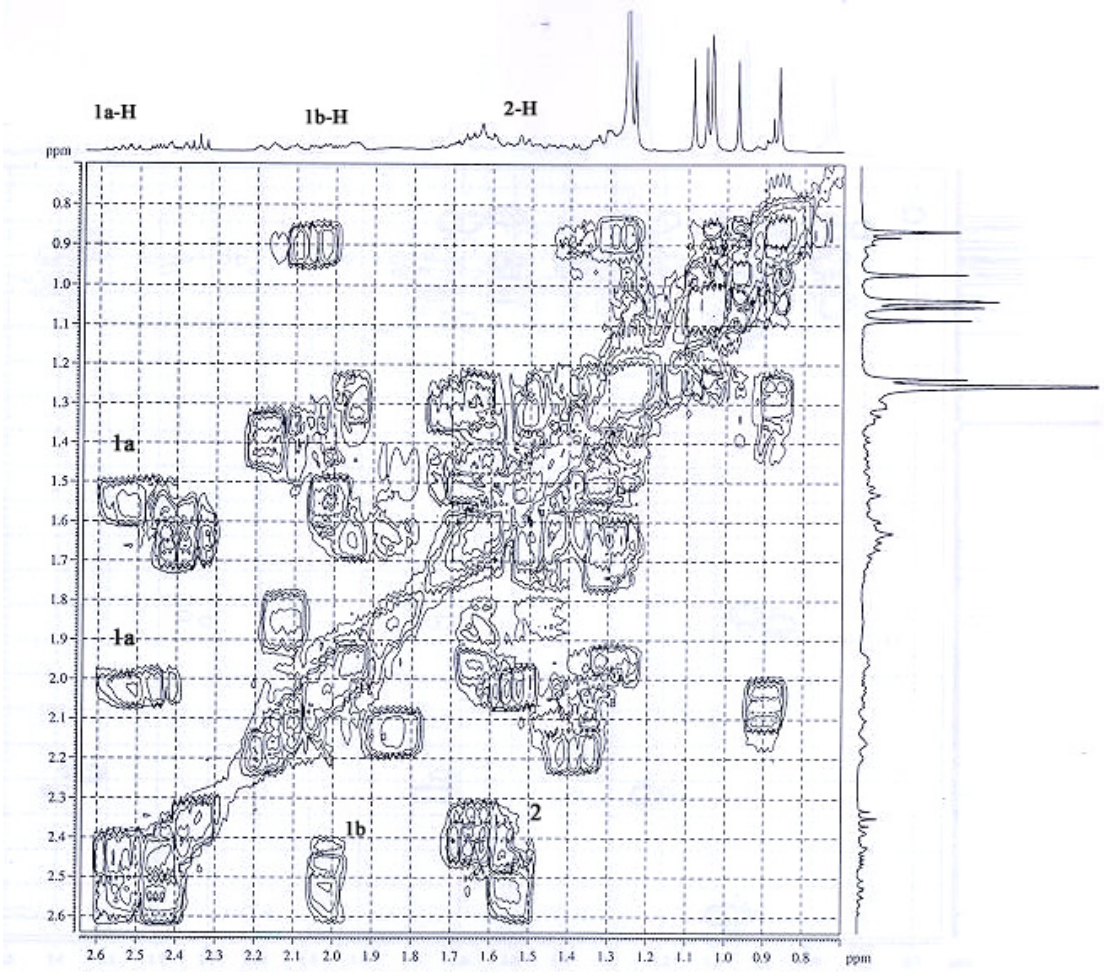

Fig. 14S H-H COSY spectrum of **2** in $CDCl_3$.

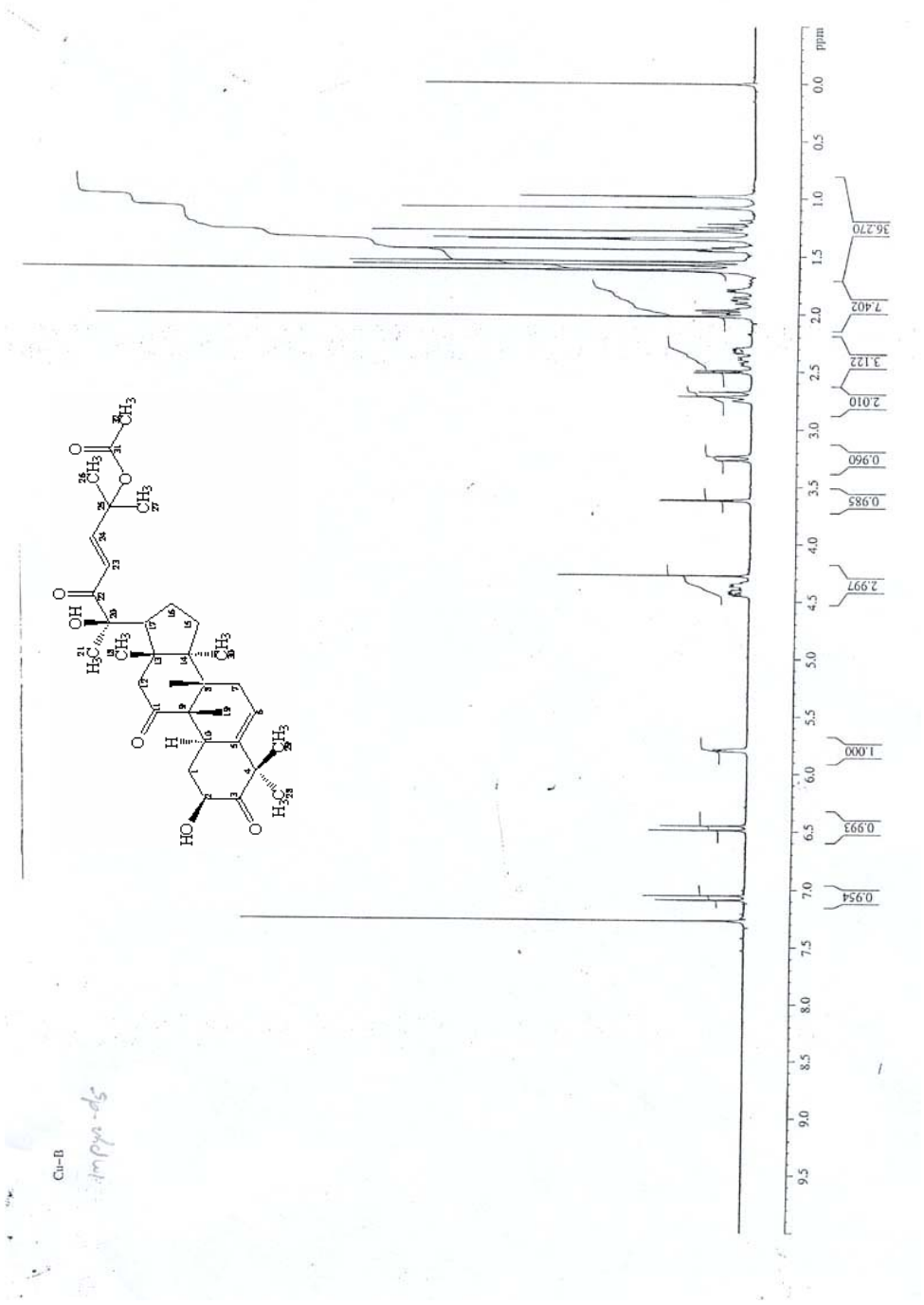

Fig. 15S 400-MHz ^{1}H-NMR spectrum of compound **3** in $CDCl_3$.

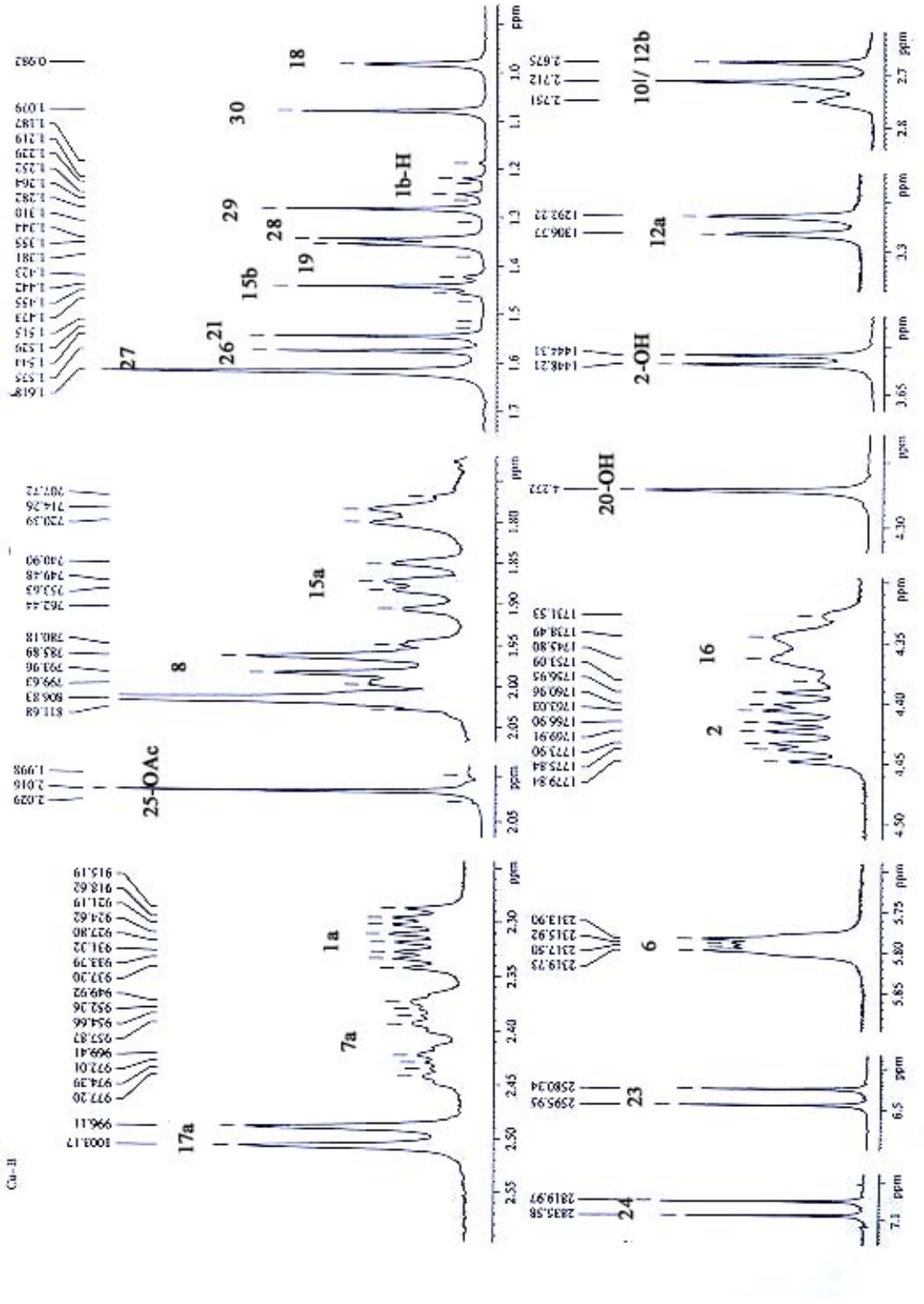

Fig. 16S Expanded 400-MHz ^{1}H-NMR spectrum of compound **3** in CD_3OD.

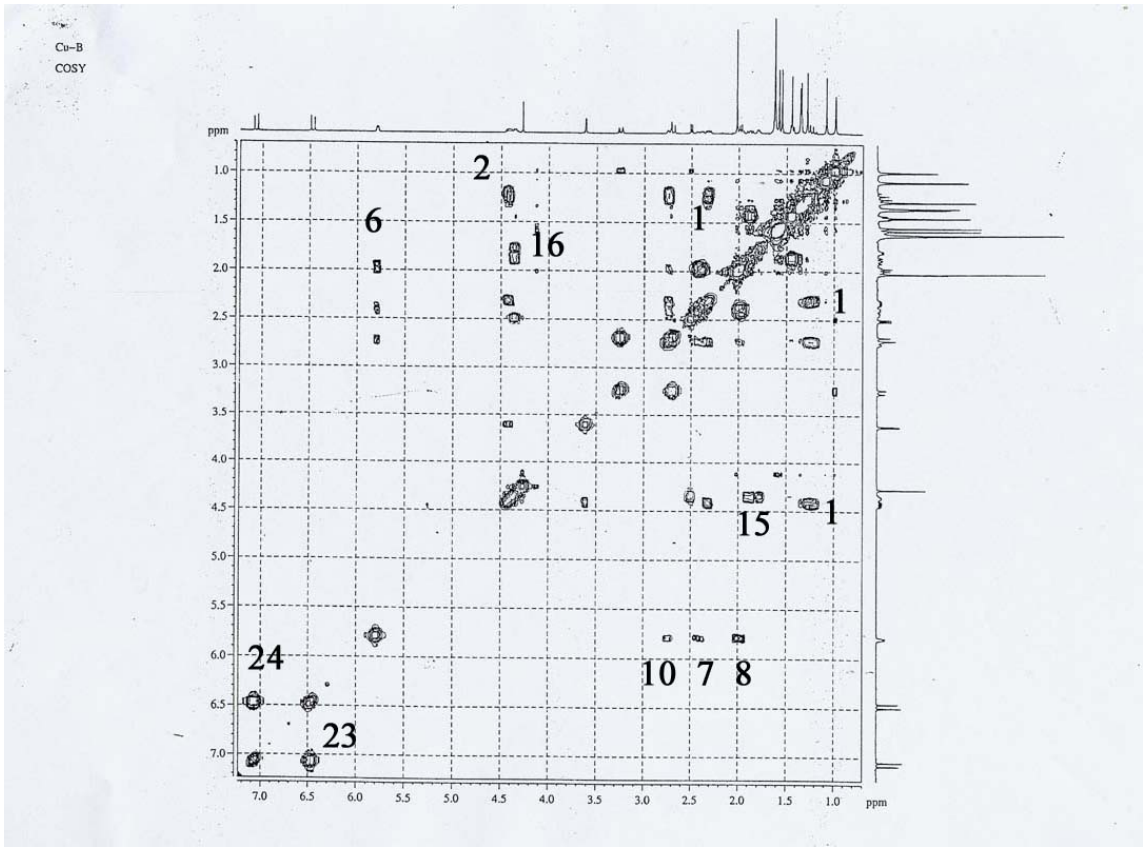

Fig. 17S H-H COSY spectrum of compound **3** in $CDCl_3$.

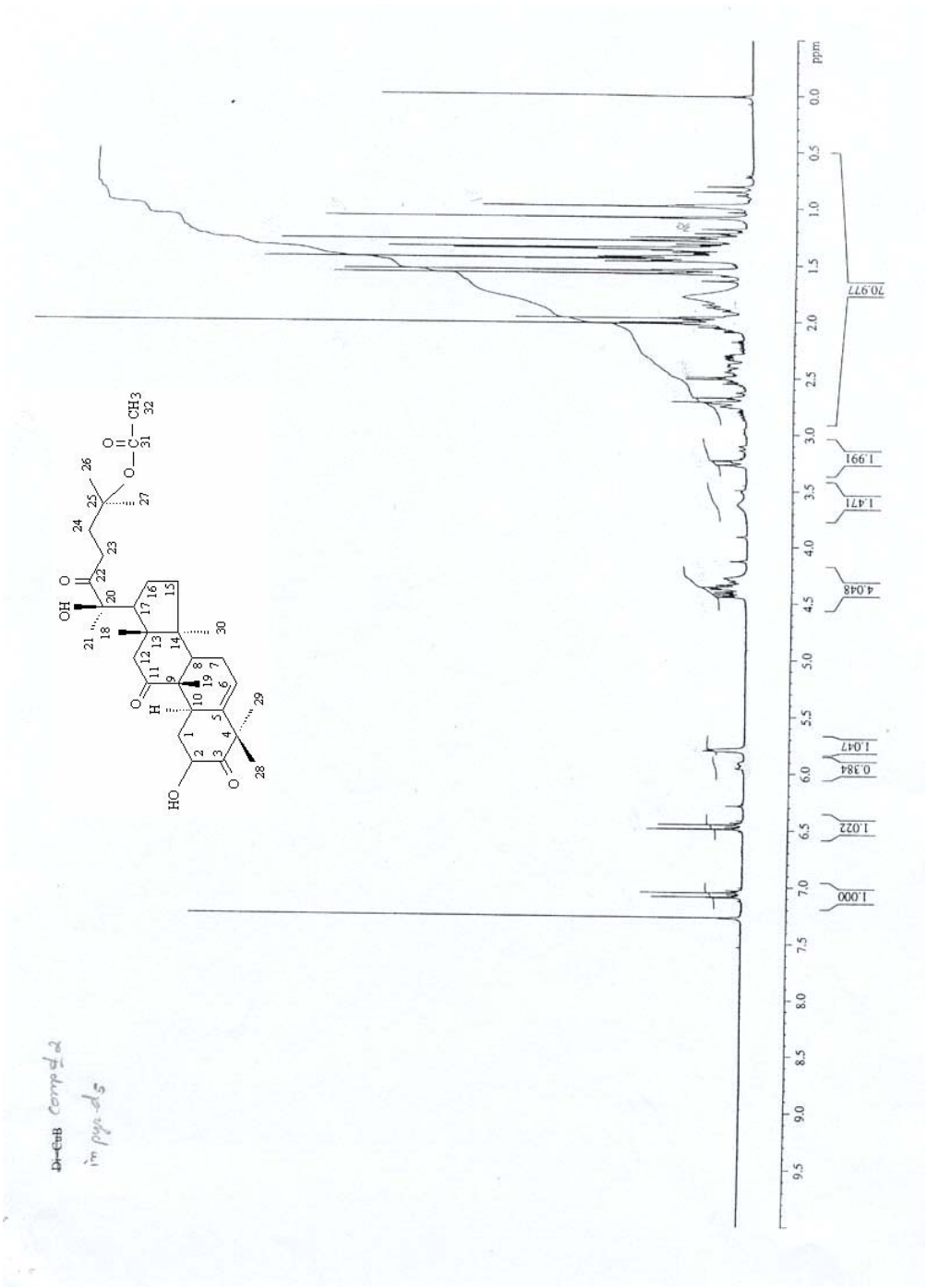

Fig. 18S 400-MHz ^{1}H-NMR spectrum of compound **4** in $CDCl_3$.

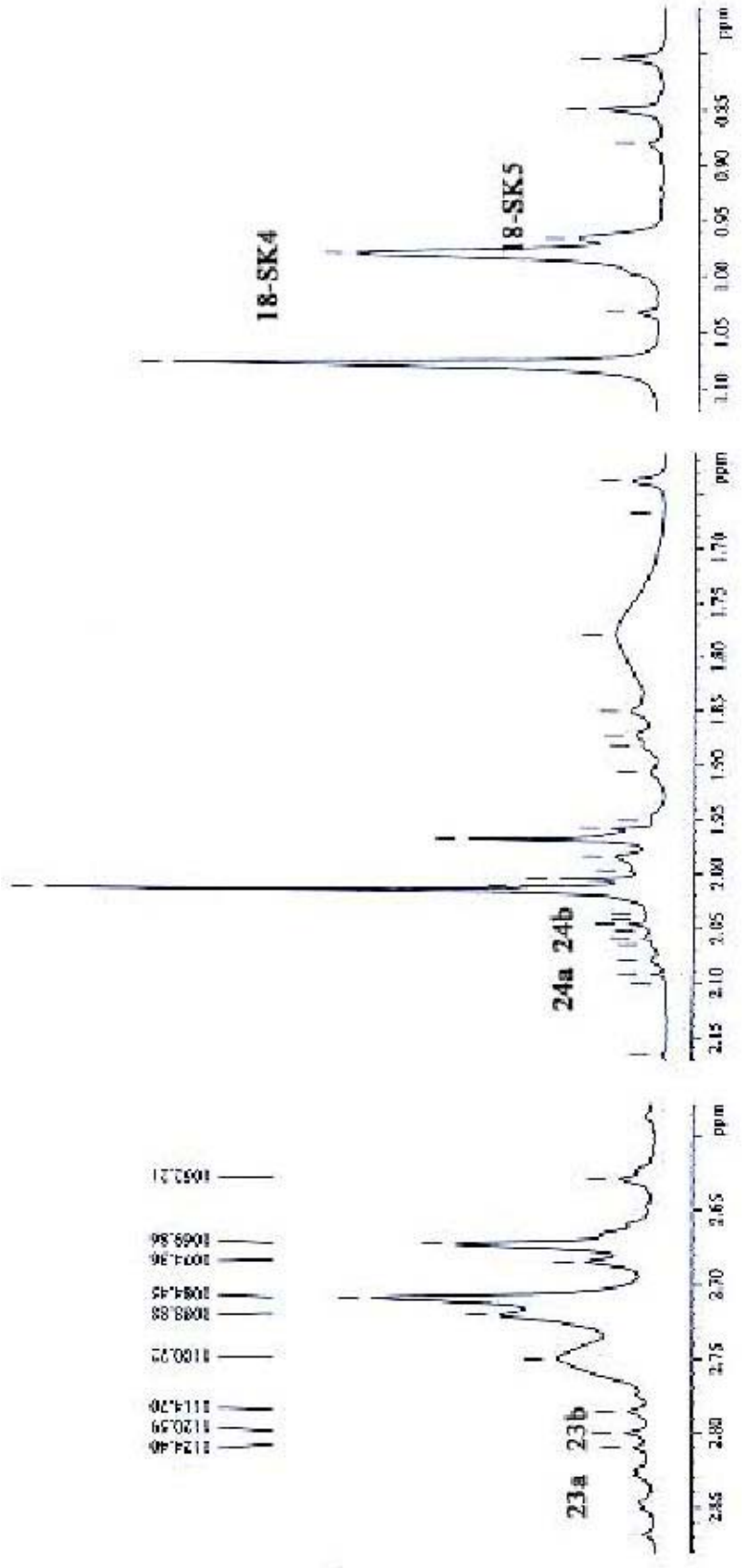

Fig. 19S Expanded 400-MHz ^{1}H-NMR spectrum of compound **4** in CD_3OD.