Imperial College London	Department of Chemistry Third Year Advanced Practical Organic Chemistry

Experiment 3

The Penicillin-Cephalosporin Conversion

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EXPERIMENT 3:

THE PENICILLIN–CEPHALOSPORIN CONVERSION

Aims of the experiment

To use chromatographic techniques to follow a reaction and to purify the products.

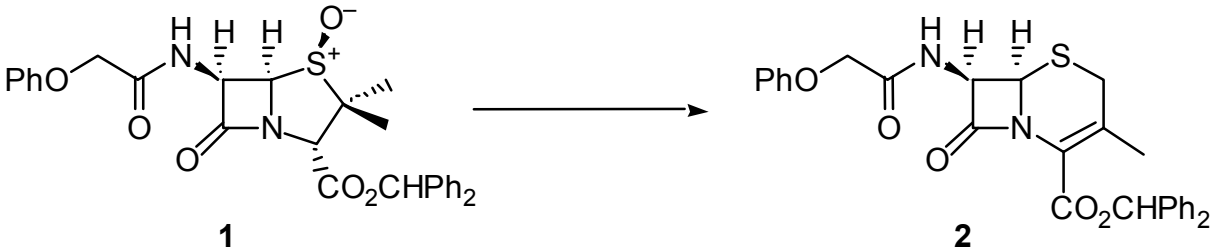
Techniques used/learned:

Flash chromatography for small scale purifications; analysis of complex nmr spectra.

Introduction

The routine purification of organic compounds, especially in large quantities, was originally carried out by tedious long column chromatography. Good separations often requires prolonged elution with solvents of low polarity. Nowadays, the technique of ***flash chromatography***[1] has become almost universal for bench-top separations. Flash chromatography involves the purification of an organic (or inorganic) compound by partition between a finely divided stationary phase, usually a specially manufactured grade of silica gel, and a rapidly moving organic solvent. The technique is highly attractive in that separations are rapid (10-20 min is possible), resolution of similar compounds is often excellent, and the technique reasonably inexpensive. In many cases reasonably unstable compounds, such as diazoketones, can be purified easily by this technique. The choice of eluant is easily found by prior testing by thin layer chromatography (t.l.c.).

In this experiment, the rearrangement[2] of the penicillin **1** to the cephalosporin **2** is conveniently followed by t.l.c. and the product isolated by flash chromatography.



1 **2**

HAZARD DATA - READ THIS BEFORE GOING ANY FURTHER!

DICHLOROMETHANE:

HARMFUL BY INHALATION, INGESTION, AND SKIN CONTACT
POSSIBLE RISK OF IRREVERSIBLE EFFECTS
IRRITATING TO EYES

Colourless volatile liquid with chloroform-like odour; b.p. 40°C; immiscible with water. Avoid breathing vapour. Avoid contact with skin and eyes. MEL 350 mgm^{-3}.

Toxic effects:	The vapour irritates the eyes and respiratory system and may cause headaches and nausea; high concentrations may result in cyanosis and unconsciousness. The liquid irritates the eyes. Poisonous if taken by mouth.
Hazardous reactions:	Mixtures with Li, Na, N_2O_4, HNO_3 liable to *explode*.
Spillage & disposal:	Wear goggles, gloves, and laboratory coat. Absorb bulk quantities on sand, shovel into buckets. Wash site of spillage with water and detergent. Dichloromethane should be placed in chlorinated waste containers for central disposal

DIETHYL ETHER:

HARMFUL BY INHALATION, AND INGESTION
MAY FORM EXPLOSIVE PEROXIDES
EXTREMELY FLAMMABLE

Colourless highly volatile liquid with characteristic odour; b.p. 34°C; immiscible with water. Liable to form explosive peroxides on exposure to light/air. Peroxides removed by treatment with aqueous sodium metabisulfite. Avoid breathing vapour. OEL 1200 mgm^{-3}.

Toxic effects:	Inhalation of the vapour may cause drowsiness, dizziness, mental confusion, faintness and, in high concentrations, unconsciousness. Ingestion may also produce these effects. Continued inhalation of low concentrations may cause loss of appetite, dizziness, fatigue and nausea.
Fire Hazard	Flash point –45°C, ignition temp. 180°C; extinguish fire with CO_2.
Hazardous reactions	Peroxide formation can result in subsequent explosion. Powerful oxidants can cause explosion. Reacts vigorously with sulfuryl chloride.
Spillage & disposal	Clear area, shut off all sources of ignition. Organise effective ventilation and allow to evaporate. Diethyl ether should be placed in the non-chlorinated waste container for central disposal

1,4-DIOXAN:

HARMFUL BY INHALATION, INGESTION, AND SKIN CONTACT
MAY FORM EXPLOSIVE PEROXIDES
HIGHLY FLAMMABLE

Colourless almost odourless liquid; b.p. 101°C; miscible with water. Liable to form explosive peroxides on exposure to light/air. Peroxides removed by treatment with aqueous sodium metabisulfite. Irritating to eyes. Avoid breathing vapour. OEL 90 mgm^{-3}.

Toxic effects:	The vapour irritates nose and eyes and this may be followed by headache and drowsiness. High concentrations may also cause nausea and vomiting, while injury to the liver and kidney is possible. Similar effects when taken by mouth.
Fire hazard	Flash point 12°C; ignition temp. 180°C; extinguish fire with CO_2.
Hazardous reactions:	Explosive peroxides formed on exposure to air/light. Reacts explosively with Raney Ni at >210°C. Addition complex with SO_3 explodes on storage.
Spillage & disposal	Clear area, shut off all sources of ignition. Wear goggles, gloves, and laboratory coat. Mop up with plenty of water and run to waste. Organise effective ventilation and evaporate remaining liquid. 1,4-Dioxan should be placed in the non-chlorinated waste container for central disposal.

ETHYL ACETATE:

HIGHLY FLAMMABLE
IRRITATING TO EYES AND RESPIRATORY SYSTEM

Colourless liquid with fragrant odour; b.p. 77°C; slightly soluble in water. Avoid breathing vapour. Avoid eye contact. OEL 1400 mgm^{-3}.

Toxic effects: The vapour may irritate the eyes and respiratory system. The liquid irritates the eyes and mucous surfaces. Prolonged inhalation may cause kidney and liver damage.

Fire hazard: Flash point –4.4°C; ignition temp. 427°C; extinguish fire with CO_2.

Spillage & disposal: Clear area, shut off all sources of ignition. Wear face shield goggles and gloves. Absorb bulk quantities on sand, shovel into buckets. Wash site of spillage with water and detergent. Ethyl acetate should be placed in the non-chlorinated waste container for central disposal.

PHOSPHORIC ACID:

CORROSIVE
CAUSES BURNS

Colourless viscous liquid, miscible with water. Avoid eye contact. OEL 2 mgm^{-3}.

Toxic effects: The liquid burns the eyes and skin severely. If taken by mouth, there would be severe internal damage.

Spillage & disposal: Wear goggles, gloves, and laboratory coat. Carefully spread sodium carbonate liberally over the spillage and mop up cautiously with plenty of water. Run to waste with running water.

PYRIDINE:

HARMFUL BY INHALATION, INGESTION, AND SKIN CONTACT
IRRITATING TO EYES, SKIN AND RESPIRATORY SYSTEM
HIGHLY FLAMMABLE

Colourless liquid with a sharp penetrating odour; b.p. 115°C; miscible with water. Avoid breathing vapour. Avoid contact with skin and eyes. OEL 2 mgm^{-3}.

Toxic effects: The vapour irritates the respiratory system and may cause headache, nausea, giddiness and vomiting. The vapour and liquid irritates the eyes and may cause conjunctivitis. the liquid may irritate the skin and cause dermatitis. Affects the central nervous system if taken by mouth and large doses act as a heart poison.

Fire hazard: Flash point 20°C, ignition temp. 482°C; extinguish fire with CO_2.

Hazardous reactions: Complex with CrO_3 unstable and decomposes violently when dry. Reacts violently with N_2O_4.

Spillage & disposal: Clear area, shut off all sources of ignition. Wear goggles, gloves, and laboratory coat. Mop up with plenty of water and run to waste. Organise effective ventilation and evaporate residual liquid.

Experimental Procedure

Preliminaries

1. Obtain dioxane (pre-dried over sodium, 20ml) from the bottle provided (**DO NOT ATTEMPT TO DRY THE SOLVENT WITH SODIUM YOURSELF!**) in a pre-dried flask.
2. Establish a t.l.c. system for the □-lactam ester **1** to give an R_f value between 0.2 and 0.3. Use a mixtures of ethyl acetate and dichloromethane. The spots can be visualised under the u.v. lamp and by placing the plates in a jar containing a few crstals of iodine.
3. Record and interpret the i.r. spectrum of the penicillin; interpret the nmr spectrum of the penicillin (provided).

Rearrangement of Penicillin V β-Sulphoxide **1***.*

In a 25ml round bottomed 2 or 3 necked flask, dissolve dry pyridine (10μl) and 85% orthophosphoric acid (14μl) in dry redistilled dioxan (see above) (10ml). Measure the acid and base using a Gilson micropipette with a ***new tip*** for each reagent. Add the penicillin ester **1** (0.50g) as provided, and heat the mixture to reflux overnight under nitrogen (***CARE***: check all joints). Before commencing heating, flush the flask with N_2 and subsequently maintain a slight positive pressure of N_2 applied to the top of the condenser, *via* a T-piece with a liquid paraffin bubbler (see Demonstrator / Technician for details). **Do not** purge the system continuously with N_2 since this will evaporate the solvent. Allow the solution to cool. Recheck the t.l.c. If necessary, adjust the solvent composition (to x% EtOAc/CH_2Cl_2) to give an R_F of ~ 0.5 for the product.

Flash Chromatography and Isolation of the Product

IMPORTANT! SAFETY NOTE: Silica gel is dangerous by inhalation. Handle the dry silica in a fume hood. Once slurried, the material can be used on the bench provided it is not allowed to run dry. Dispose of all silica residues in the bin provided.

Cover the sintered glass column with the plastic safety webbing provided and clamp it vertically over a conical flask. **IN A FUME HOOD**, slurry BDH flash chromatography silica (12g) in a conical flask with dichloromethane (CH_2Cl_2). Pour the slurry into the column and wash in the residue with more CH_2Cl_2. Using the hand bellows, force the CH_2Cl_2 through the column and thus compact the silica. It is essential that the level of solvent is not forced beyond the top of the silica otherwise channels will form in the silica and its effectiveness destroyed. The surface of the silica pad *must* be as flat as possible. With extreme care, add more CH_2Cl_2 to the column; do not disturb the silica surface. Pouring the solvent carefully down the side of the column over the blade of a wide spatula is helpful. Again, force the solvent through the column so that the surface of the silica is just damp with CH_2Cl_2. If cracking or channelling occurs, *repack the column*.

Evaporate the dioxan solution containing the product to *complete dryness*. dissolve the residue in the ***minimum*** volume of CH_2Cl_2 and carefully apply dropwise to the surface of the silica. Wash on the last traces with a further small volume of CH_2Cl_2. Using the bellows, carefully force the liquid onto the column so that the level of liquid is again just flush with the silica surface. Rinse the edges of the glass with a little more CH_2Cl_2 and again force it onto the column. Cover the top of the silica with fine sand to a depth of *ca.* 1 cm.

Fill the column with x/2 % EtOAc / CH_2Cl_2. Force the solvent rapidly through the column and collect 5 x 10ml fractions. Take care that the bung does not fly out during the operation! Further elute the column with x % EtOAC / CH_2Cl_2 (50ml). Collect a further 10 fractions and check all by t.l.c. Combine those containing the pure product. Rotary evaporate to dryness in a pre-tared flask. Check the weight of the residue. Using small volumes of CH_2Cl_2, transfer the residue *via* filtration through a Pasteur pipette containing a cotton wool plug, to a small conical flask. Evaporate (fume cupboard) to dryness and recrystallise the residue from ethyl acetate. Dry the product under vacuum overnight at room temperature. Record the yield, m.p., n.m.r. and i.r. spectra and compare with those of the starting penicillin. Transfer the product to a small labelled sample tube.

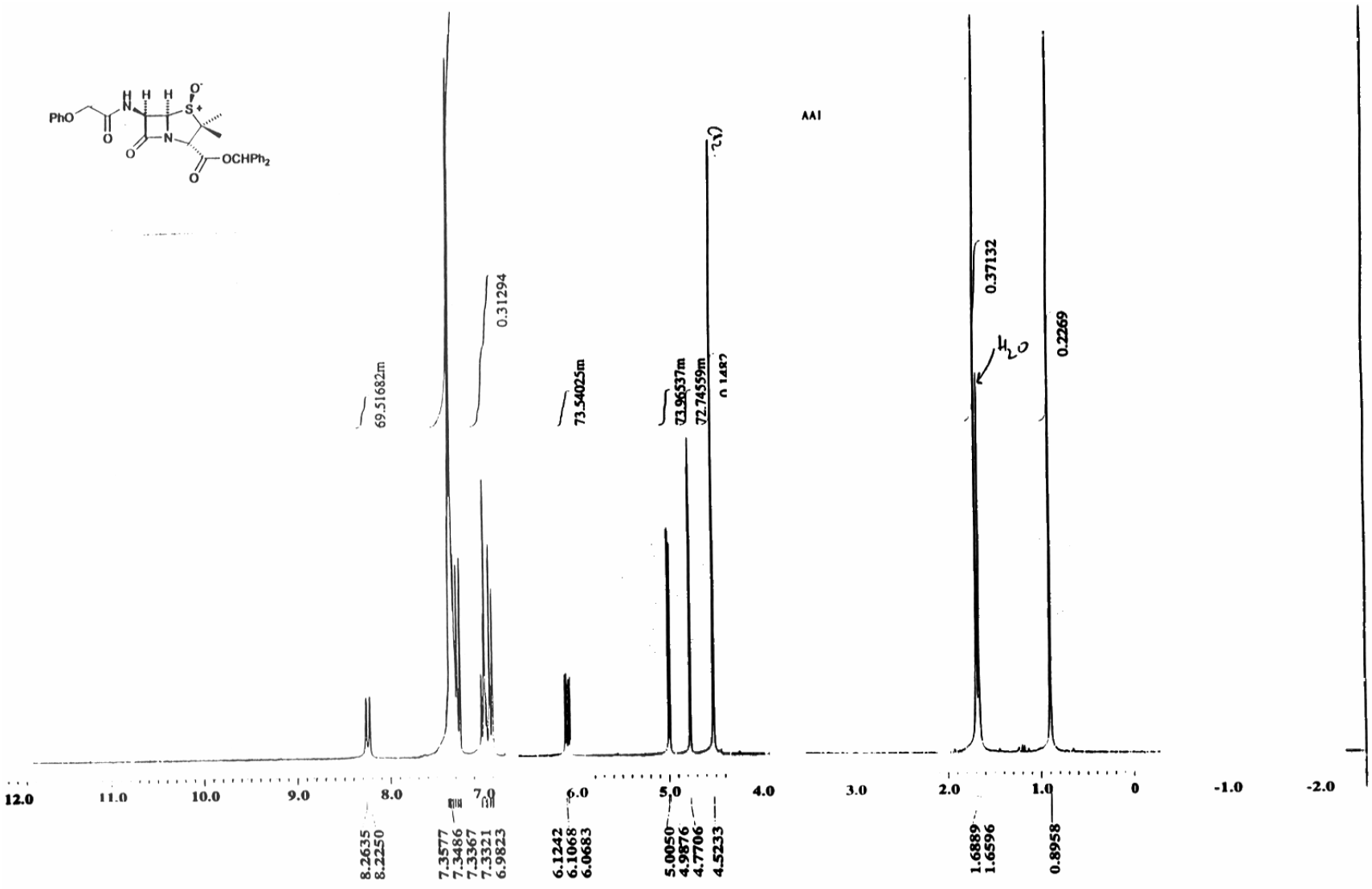
Write Up

The write up should conform to *Organic and Biomolecular Chemistry* style (Title, Abstract, Introduction, Results and Discussion, Experimental, References). Your introduction should include discussion of the following points:

1. A full arrow pushing mechanism for the transformation you have carried out.
2. One recent (2003 or later) example from the primary literature of the preparation of β-lactams.
3. A brief discussion of the antibiotic mode of action of β-lactams.

References and Notes

1. W.C. Still, M. Kahn and A. Mitra, *J. Org. Chem.*, 1978, **43**, 2923.
2. R.D.G. Cooper and D.O. Spry, 'Cephalosporins and Penicillins. Chemistry and Biology', Ed. E.H. Flynn, Academic Press, New York, 1972, p. 183;
3. P.G. Sammes, *Chem. Rev.*, 1976, **76**, 113 and references cited therein.



X : parts per Million : 1H

Peak listing for data 1d_1H_spectrum.17

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2:	8.2250	2222.1320	2815.9959
3:	7.3861	1995.4939	2878.0122
4:	7.3797	1993.7619	3280.6713
5:	7.3687	1990.7929	9433.3924
6:	7.3651	1989.8032	11070.4106
7:	7.3577	1987.8238	14011.3680
8:	7.3486	1985.3496	32623.6980
9:	7.3367	1982.1331	25888.7840
10:	7.3321	1980.8960	27211.5282
11:	7.3120	1975.4527	9286.5541
12:	7.3037	1973.2260	5874.6447
13:	7.2845	1968.0301	8796.3130
14:	7.2799	1966.7930	8303.9106
15:	7.2717	1964.5662	2238.7468
16:	7.2598	1961.3497	3115.1091
17:	7.2524	1959.3704	7607.4345
18:	7.2451	1957.3910	9091.3860
19:	7.0207	1896.7727	3790.1417
20:	7.0171	1895.7830	2563.6788
21:	6.9933	1889.3501	6494.0448
22:	6.9823	1886.3810	12799.9749
23:	6.9694	1882.9171	2031.5865
24:	6.9658	1881.9274	2933.3667
25:	6.9356	1873.7625	7853.8518
26:	6.9319	1872.7728	9734.5759
27:	6.9237	1870.5460	2642.5162
28:	6.9072	1866.0925	4323.4454
29:	6.9035	1865.1028	7673.9163
30:	6.8998	1864.1131	6530.5377
31:	6.1242	1654.5471	3751.6662
32:	6.1068	1649.8461	3806.8281
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34:	6.0683	1639.4544	3643.9222
35:	5.0050	1352.1980	10349.0112
36:	4.9876	1347.4970	9669.2350
37:	4.7706	1288.8581	14655.2975
38:	4.5233	1222.0543	28529.3025
39:	1.6889	456.2848	34410.7205
40:	1.6596	448.3673	17636.0491
41:	0.8958	242.0178	37797.5770

Integral listing for data 1d_1H_spectrum.17

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3:	7.1059	6.8403	436528975.5922	0.3129	13.321
4:	6.1809	6.0161	102584768.1095	0.0735	3.131
5:	5.0673	4.9318	103177800.0724	0.0740	3.149
6:	4.8429	4.7111	101476260.7389	0.0727	3.097
7:	4.6131	4.4528	206732796.2762	0.1482	6.309
8:	1.8089	1.5772	517965140.2645	0.3713	15.807
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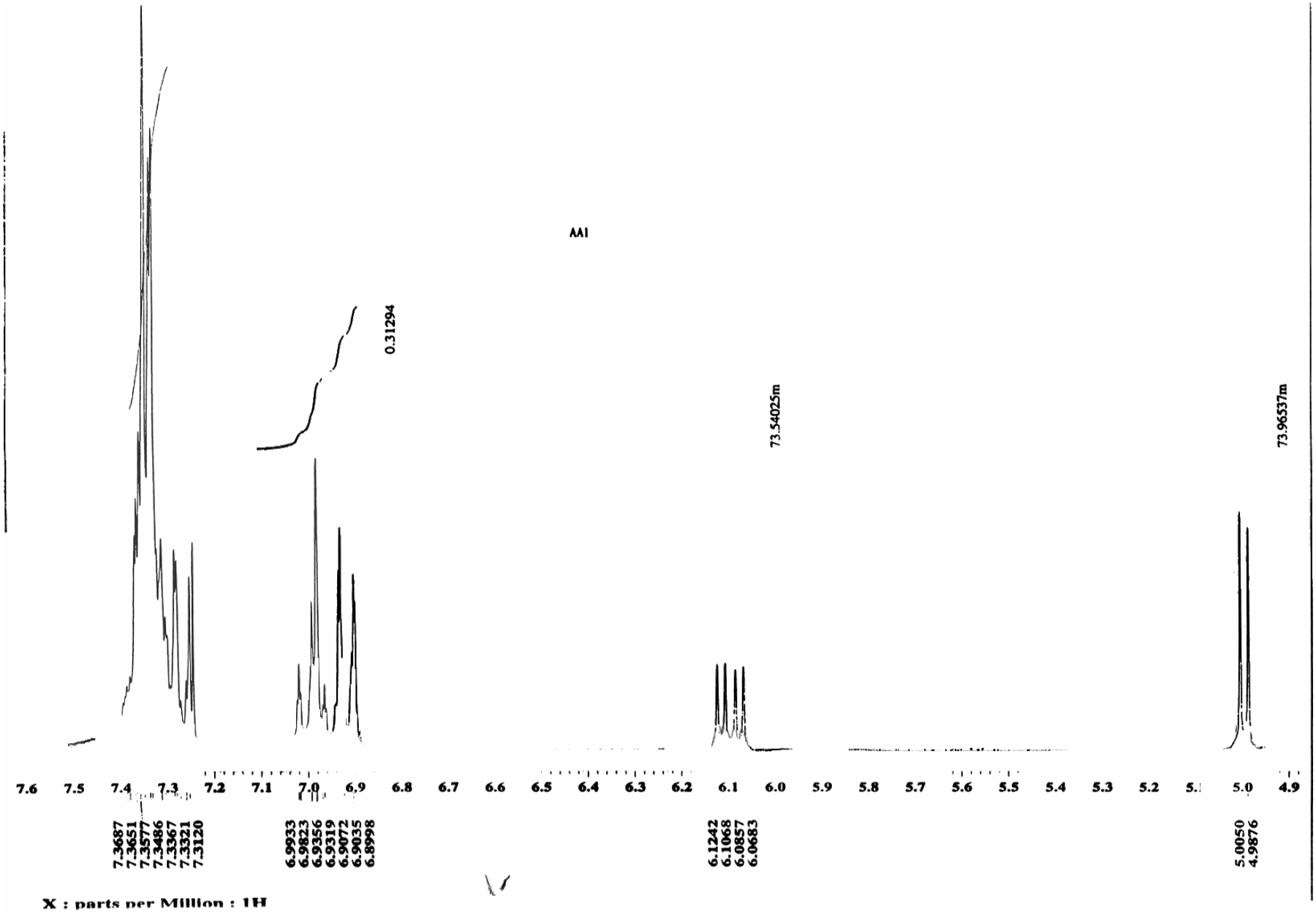
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73.96537m
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7.3687
7.3651
7.3577
7.3486
7.3367
7.3321
7.3120
6.9933
6.9823
6.9356
6.9319
6.9072
6.9035
6.8998
6.1242
6.1068
6.0857
6.0683
5.0050
4.9876
X : parts per Million : 1H