

SYNTHESIS, ANTIMICROBIAL ACTIVITIES OF 8-METHOXYCOUMARIN ANALOG OF CHLORAMPHENICOL AND MASS SPECTRAL FRAGMENTATIONS OF 2-DICHLORO-ACETAMIDO-3-HYDROXY-1-[3-(8-METHOXYCOUMARINYL)]-1-PROPANONE

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ABSTRACT

The title compound is prepared as a potential antimicrobial agent by adopting Sorm's method. The fragmentation of 2-dichloroacetamido-3-hydroxy-1-[3-(8-methoxycoumarinyl)]-1-propanone (5) under electron impact is discussed. The compounds (3-6) are subjected to *in vitro* antibacterial and antifungal activities.

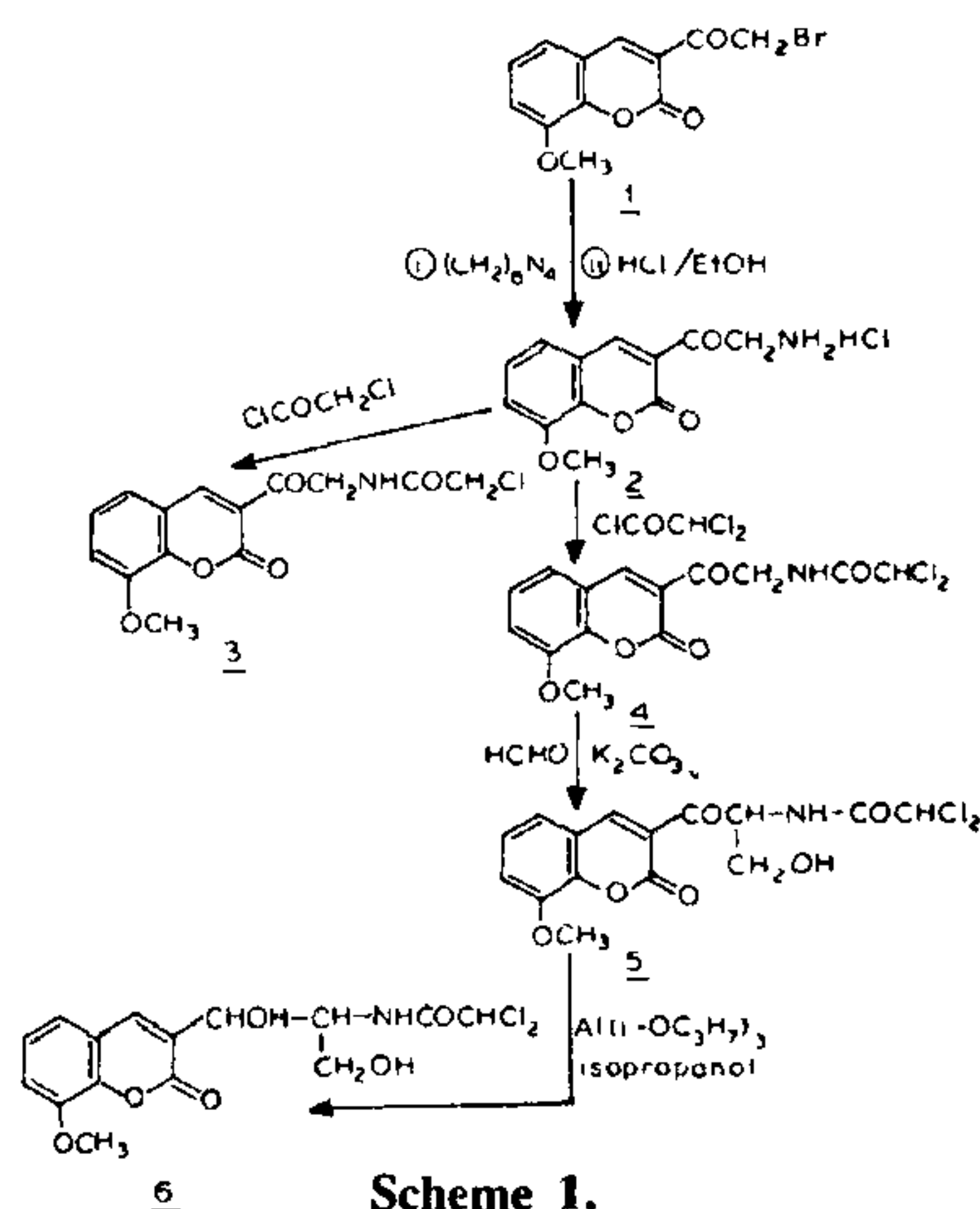
INTRODUCTION

THE chemical stability of chloramphenicol and the ease with which 2-aminopropane 1,3-diol side-chain can be attached to various aromatic systems have made this antibiotic, a popular candidate for modification study. As part of our interest in the synthesis of various analogs and heterocyclic analogs of chloramphenicol¹⁻⁷, we report in the present investigation, the preparation and antimicrobial activities of 8-methoxycoumarin analog of chloramphenicol with a view to probing on the unsolved problem of the structure-activity relationship.

MATERIALS AND METHODS

8-Methoxy-3-bromoacetyl coumarin (1) obtained by the bromination of 8-methoxy-3-acetyl coumarin⁸ was converted into hexamethylene salt in dichloro methane solution. This was further converted into aminehydrochloride (2) by an earlier procedure⁴. The aminehydrochloride (2) was converted to chloroacetamide (3) by treating it with chloroacetyl chloride and to dichloroacetamide (4) as described earlier⁵. However the hydroxymethylation carried out in the mixture of paraformaldehyde and potassium carbonate in methanol, yielded crystalline 2-dichloroacetamido-3-hydroxy-1-[3-(8-methoxycoumarinyl)]-1-propanone (5). This, on modified Meerwein-Ponndorf-Verley reduction method⁹, gave red oil which on repeated crystallizations either with benzene or ethylene chloride yielded the desired chloramphenicol (6) (scheme 1).

The melting points of the new compounds were determined by the capillary method in an open



Scheme 1.

paraffin bath and were uncorrected. IR spectra were recorded on a Perkin-Elmer Spectrophotometer (ν_{max} in cm^{-1}). PMR spectra were recorded on Varian A60 (90 MHz) spectrometer in DMSO-d_6 using TMS as the internal reference (chemical shifts in δ ppm). Mass spectrum was recorded on Finnigan-4121 G-C mass spectrometer using direct inlet system at an ionization potential of 70 eV.

RESULTS AND DISCUSSION

The results of elemental analysis agree with theoretical values within the limits of experimental error. The new compounds were also confirmed by their IR and PMR data.

In support of the mass spectral fragmentation pattern suggested for benzofuran and coumarin

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analogs of chloramphenicol and their precursors^{4,6}, it was thought desirable to confirm the structure of 2-dichloroacetamido-3-hydroxy-1-[3-(8-methoxy-coumarinyl)]-1-propanone (**5**) by detailed mass spectral fragmentation pattern.

Similar to the earlier mass spectra⁴, the compound **5** failed to produce molecular ion peak at an ionization potential of 70 eV. Because of its larger side-chain the molecule can split in three different ways.

The principal fragmentation occurs by the homolytic cleavage of central C-C bond at the ketone carbonyl group to give the most abundant even electron ion **7** which constitutes a base peak at m/z 203 together with ion **8** (m/z 170) consisting of side-chain. Ion **7** may then undergo ring contraction by losing a molecule of carbon monoxide from the lactone carbonyl group to give ion **9** which undergoes further fragmentation to end up with ion **11** as described earlier⁵.

Another major fragmentation occurs by McLafferty-rearrangement involving the transfer of hydrogen atom from methylene group to π bond of the aromatic pyrone ring to give the odd electron ion **12** at m/z 204. Ion **12** on further fragmentation produces ion **14**. Ion **14** on losing a hydrogen atom produces the ion **10**.

The third major fragmentation in ion **5** occurs by another type of McLafferty-rearrangement involving the transfer of hydrogen atom from the hydroxyl

group to oxygen atom of the ketone carbonyl group to give ion **15** at m/z 343. In ion **15** the homolytic cleavage of central C-C bond at the ketone carbonyl group produces ion **7** together with ion **16** at m/z 140. Similarly ions **17**, **18** and **19** are produced. Furthermore ion **19** ($M-H_2O^+ -CHCl_2^+$) is seen at m/z 272 (scheme 2).

All the compounds (**3-6**) have been screened for their *in vitro* antibacterial activity by the cup-plate method¹⁰. Chloramphenicol was used as the standard drug. Compounds **3** and **4** were inactive towards *E. coli* but showed activity against *S. aureus*, *S. sonnei* and *Bac. cirroflagellosus*. However, compound **5** showed little activity against *E. coli* but its activity remained unchanged towards *S. aureus* and *S. sonnei* as compound **4**. But the activity of compound **6** increased towards *E. coli* but remained the same for other bacteria. The antifungal activity was carried out by the turbidity method¹¹. Compound **3** showed better inhibition. But the extent of inhibition decreased for compound **4**. Compound **5** is less active; but the analog (final product) showed greater inhibition towards all the fungi.

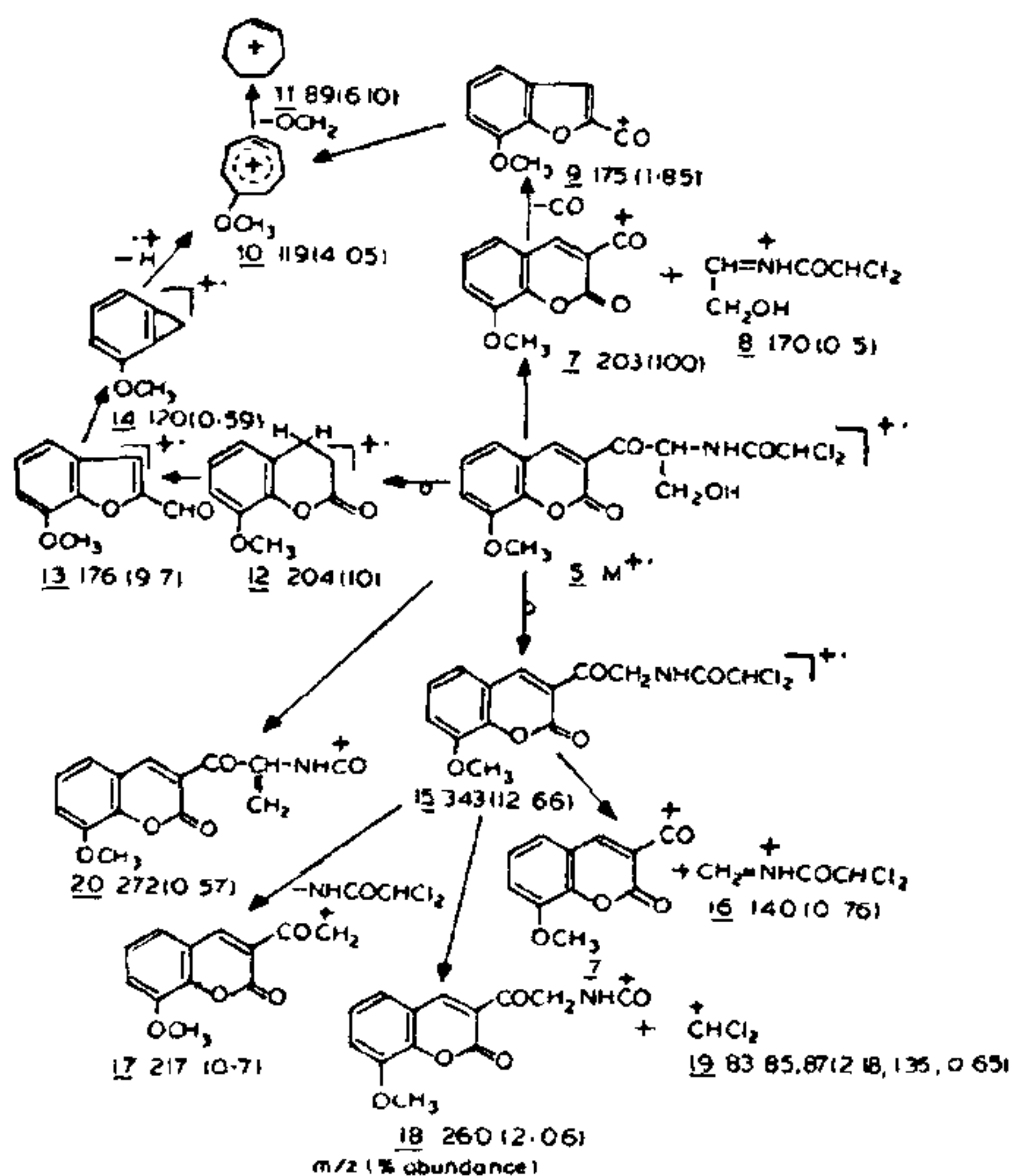
EXPERIMENTAL PROCEDURE

8-Methoxy-3-bromoacetyl-coumarin (**1**) was prepared according to the known method⁸.

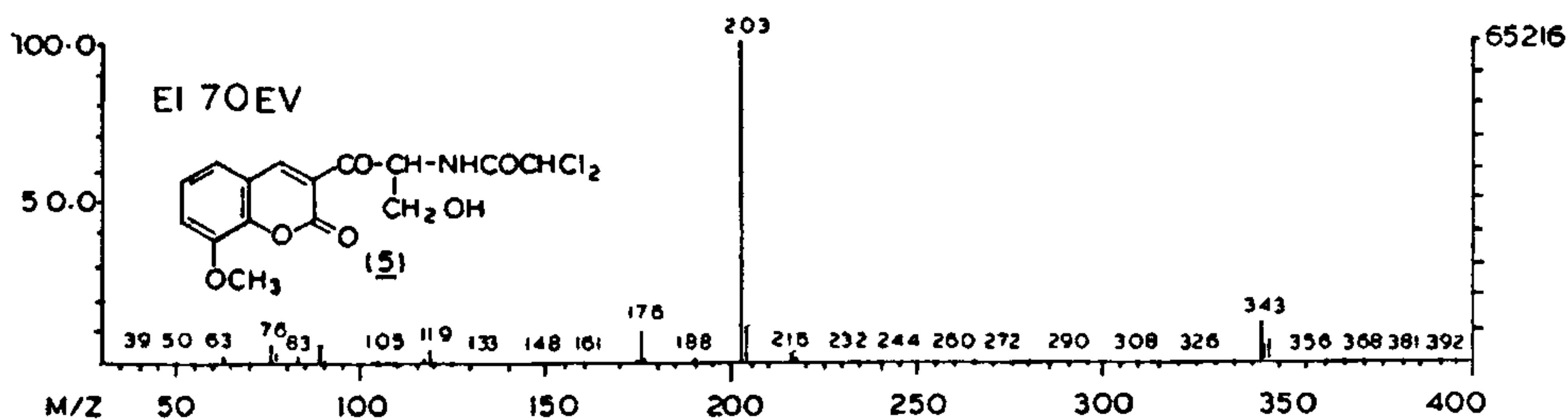
Aminomethyl-3-(8-methoxycoumarinyl) ketone hydrochloride (**2**): To the stirred mixture of powdered anhydrous hexamethylene tetramine (16.8 g; 0.12 mol) in dichloromethane (200 ml) was added a clear solution of 8-methoxy-3-bromoacetyl-coumarin (29.7 g; 0.1 mol) in dichloromethane (300 ml). Immediately a bright yellow solid separated out, which was stirred at room temperature for 2 hr, cooled and filtered. Yield 79–80% (35 g) m.p. 205–207° (d).

The hexamine adduct (43.7 g; 0.1 mole) was stirred in a solution of concentrated hydrochloric acid (50 ml) and ethyl alcohol (100 ml) for 3–4 hr at room temperature. The mixture was cooled to 0° and then filtered. The resulting solid was washed with dry ether to give bright yellow aminehydrochloride contaminated with paraformaldehyde and ammonium chloride. Yield (27 g) m.p. 250° (d).

Chloroacetamidomethyl-3-(8-methoxycoumarinyl) ketone (**3**): A suspension of crude aminomethyl 3-(8-methoxycoumarinyl)-ketone hydrochloride (2.69 g; 0.01 mol) in chloroacetyl chloride (9 ml) was heated and stirred at 80° for 30 min. On stirring the crystalline chloroacetamide separated out. The solid was filtered and washed with a little ethyl



Scheme 2.



acetate. Crystallization from ethyl acetate gave the product in 84% yield (2.5 g) m.p. 209–210°.

IR (Nujol) : 3250 (–NH), 1730 (C=O lactone), 1690 (C=O amide), 1665 (C=O ketone).

Dichloroacetamidomethyl-3-(8-methoxycoumarinyl)ketone (4): A suspension of crude aminomethyl 3-(8-methoxycoumarinyl)ketone hydrochloride (26.9 g; 0.1 mol) and α,α' -dichloroacetyl chloride (90 ml) was heated and stirred at 80° for about 45 min. The mixture was cooled and then poured into ice. Then the mixture was stirred till the dichloroacetamide separated out. The solid was filtered, dried and recrystallization from acetic acid gave the product in 76% yield (26 g) m.p. 220–221°.

IR (Nujol) : 3350 (NH), 1735 (C=O lactone), 1695 (C=O amide), 1665 (C=O ketone).

PMR : 3.8 (s, 3H, OCH₃), 4.4–4.5, (m, 2H, –CH₂), 6.7 (s, 1H –CHCl₂), 7.3–8.3 (m 4H, aromatic), 8.7–8.8 (m, 1H, –NH).

2-Dichloroacetamido-3-hydroxy-1-[3-(8-methoxycoumarinyl)]-1-propanone (5) : To a mixture of the dichloroacetamide (3.43 g; 0.01 mol) and paraformaldehyde (0.06 g) in methanol (5 ml) was added potassium carbonate (0.06 g). The mixture was stirred at room temperature for 2 hr. The colour of the mixture changed to red and the solid material became more dense (the mixture never became clear). The mixture was poured into ice. On stirring the colloidal suspension with a small quantity of sodium chloride, a pale yellow solid separated. The resulting solid was filtered and dried. The whole lot on fractional crystallization from benzene-ethyl acetate mixture gave the desired product in 40–41% yield (1.5 g), m.p. 200–202°.

IR (KBr) : 3450–3400 (–OH), 3300 (–NH), 1735 (C=O lactone), 1700 (C=O amide), 1690 (C=O ketone).

PMR : 3.3 (s, 1H, –OH), 3.8 (s, 3H, –OCH₃), 3.9–4 (m, 2H, –CH₂–), 4.7–4.8 (m, 1H, –CH), 6.6 (s, 1H, –CHCl₂), 7.3–8.3 (m, 4H aromatic), 8.7–8.8 (m, 1H, –NH–).

dl-threo-2-Dichloroacetamido-1-[3-(8-methoxycoumarinyl)] propane-1,3-diol (6) : A mixture of 2-dichloroacetamido-3-hydroxy-1-[3-(8-methoxycoumarinyl)]-1-propanone (3.73 g; 0.01 mol) and aluminium isopropoxide (2.35 g; 0.011 mol) in dry iso-propanol (30 ml) was heated and refluxed on steam bath for 7 hr. The iso-propanol was removed by vacuum distillation. To the remaining pale red gummy residue, aqueous sodium chloride (16 ml, 10% w/v) was added and refluxed for another 15 min. the precipitated aluminium hydroxide was removed from the hot solution by filtration and the filter cake washed thoroughly with several portions of ether. The combined ether filtrates and the ether extracts of the original aqueous filtrate were combined and dried over sodium sulphate. Removal of ether under reduced pressure yielded a red oil which on recrystallizations either with benzene or ethylene chloride produced the desired product in 27% yield, (1 g), m.p. 160–161°.

IR (Nujol), 3500–3350 (–OH), 3250 (–NH), 1730 (C=O lactone), 1690 (C=O amide)

PMR : 3.3 (s, 1H, –CH₂OH), 3.4–3.7 (m, 1H, –CH(OH)–1), 3.8 (s, 3H, –OCH₃), 3.9–4 (m, 2H, –CH₂–OH), 4.5–4.8 (m, 1H, Ch–NH), 6.0–6.1 (m, 1H, –CH(OH)), 6.6 (s, 1H, –CHCl₂), 7.3–8.3 (m, 4H, aromatic), 8.7–8.8 (m, 1H –NH–).

Evaluation of antibacterial activity:

Antibacterial activity of the test compounds (3–6) was carried against *Escherichia coli*, *Bacillus cirroflagellosus*, *Staphylococcus aureus*, and *Shigella sonnei* by the cup-plate method¹⁰. In this method a 10 mm diameter cup was made impregnated with

Table 1 Antimicrobial activities of the compounds (3-6)

Antibacterial activity					Antifungal activity			
Zone of inhibition (mms)					Inhibition in per cent transmission			
Comp. No.	Es ^a	Bc ^a	S.aur ^a	Ss ^a	C.alb ^b	A.ni ^b	A.fl ^b	
3	-	28	18	12	57	52	78	
4	-	25	24	20	50	37	34	
5	17	24	20	20	31	37	45	
6	23	24	20	20	45	46	48	
Chlor- amphenicol	30	35	31	32	Salicylic acid	20	70	25

'-' Indicates no inhibition; a: Es - *Escherichia coli*; Bc - *Bacillus cirroflagellus*; S.aur - *Staphylococcus aureus*; Ss - *Shigella sonnei*; b: C.alb - *Candida albicans*; A.ni - *Aspergillus niger*; A.fl - *Aspergillus flavus*.

test compound (100 µg/0.1 ml of dimethylformamide) on an agar plate seeded with the above mentioned organisms and measuring the diameter of zone of inhibition for 24 hr at 37°

Evaluation of antifungal activity:

Antifungal activity of the compounds was carried against *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus* by turbidity method¹¹. The method consists of adding 100 µg/0.1 ml of the test compound into the test tubes containing the above mentioned fungi. These test tubes were shaken for 48 hr. The extent of inhibition was then determined by measuring the decrease in turbidity in terms of per cent transmission at 660 nm. Salicylic acid was (5% w/v) used as a positive control. Their results are also presented in table 1.

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