CHAPTER—IV

PART — I

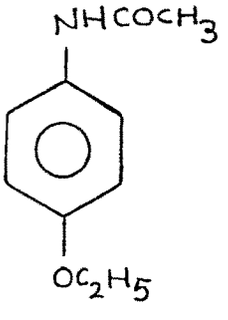
SYNTHESIS OF ANILIDES, AMIDES AND SULFONAMIDES OF COUMARIN DERIVATIVES

CHAPTER - IV

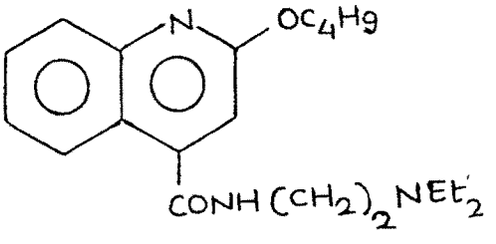
PART - I : SYNTHESIS OF ANILIDES AND AMIDES OF COUMARIN DERIVATIVES :

INTRODUCTION

Anilides and amides are known to have diverse physiological activity. Phenacetin (1) is proven analgesic and antipyretic. 2-Butoxy nupercaine (2), potent anaesthetic and a narcotic drug, was discovered while searching for other compounds as antipyretics in the acetanilide series. Other compounds such as oxindole (3) and its ring homologues and dihydrocarbostynil (4) were also examined for their activity as local anaesthetics.



(1)



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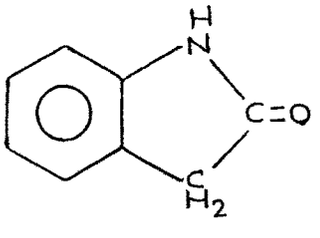

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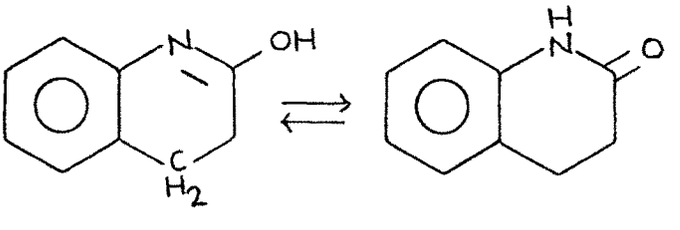
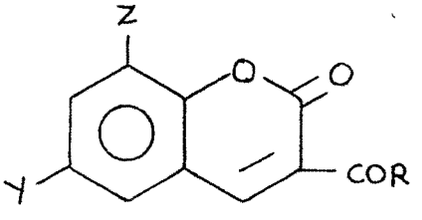


(4)

A number of publications in recent years suggested that anilides and amides of coumarin derivatives have been found to have antibacterial and antifungal activity. Some of them have been briefly mentioned here.

Werder[1,2] reported the sedative and toxic properties of N,N-diallyl coumarin-3-carboxamides. R.O. Clinton and S.C. Laskowski[3] synthesised simple and substituted coumarin-3-carboxamide (5) from coumarin-3-carbonylchloride and diallyl-aminoamine. Genshan Sunagawa and Hideo Nakao[4] prepared various 8-methoxy-3-coumarin carboxamides (6).



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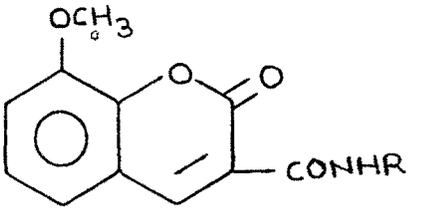
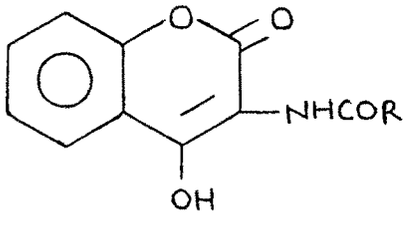


(6)

L. Reppel and W. Schmollack[5] prepared number of 3-monoacylamino-4-hydroxycoumarin (7) and N-(4-hydroxy-3-coumarinyl) urea (8) for their biological evaluation.



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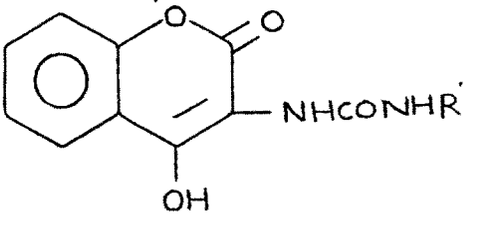
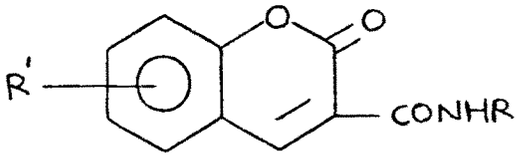


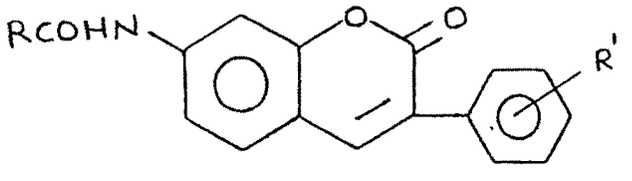
(8)

Number of carboxamides derivatives (9) had been synthesised by LIPHA[6] from 3-carbethoxy-4-hydroxy-coumarin and $nC_7H_{15}NH_2$, 4-hydroxycoumarin and $nC_7H_{15}NCO$, 4-hydroxycoumarin and Pyridine-3-carboxylic acid azide. These compounds showed antibacterial and antifungal activity.



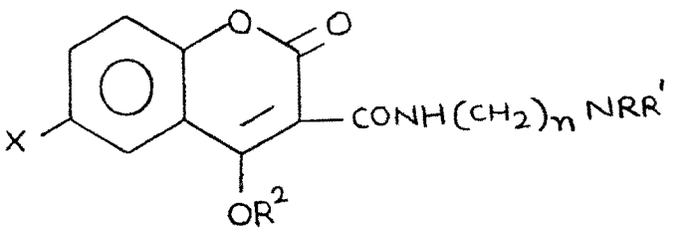
(9)

J.R. Geigy A.G.[7] synthesised coumarin carboxamide (10) from N-acylation of 3-phenyl-7-aminocoumarin or its derivatives which were used as optical brighteners.



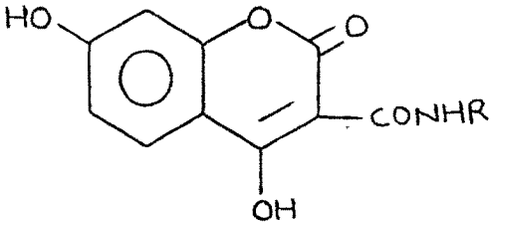
(10)

LIPHA[8] also synthesised 4-hydroxycoumarin-3-carboxylic acid-N-(aminoalkyl) amides (11) by condensing alkyl-4-hydroxy-coumarin-3-carboxylates with alkylene diamine. These compounds were used as anaesthetics and as fibrinolytic, antiinflammatory, analgesic and antitussive agents.



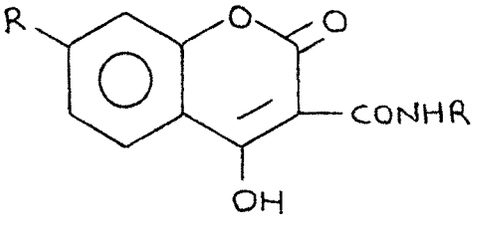
(11)

Kento Okumura et al. [9-11] reported the bactericidal activity of 3-carbamoyl-4,7-dihydroxycoumarin which were synthesised from $PhNH_2$ and Et-4,7-dihydroxy coumarin-3-carboxylate.



(12)

They also synthesised number of 3-N-substituted carbamoyl-4-hydroxycoumarin (13) and 3-alkyl carbamoyl-4-hydroxy-7-amino coumarin (14) which were useful as bactericides and antituberculous drugs.



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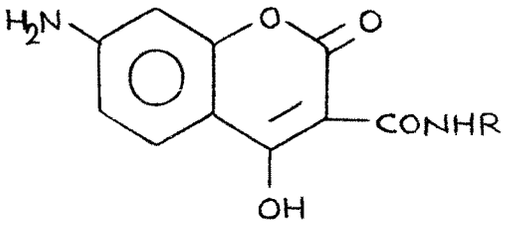
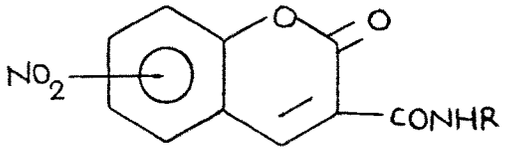


(14)

Ichikawa Masutaka and Ichibagase Hisashi[12] prepared N-substituted 6-nitro-and 7-nitro-3-coumarin carboxamides, N-(2-pyridyl)-7-nitro-8-hydroxy- and N-(2-pyridyl)-7-nitro-8-methoxy-3-coumarin carboxamides. They reported that some N-(2-pyridyl) amide and nitro furfurylidene derivatives showed strong activity against tubercle bacilli.



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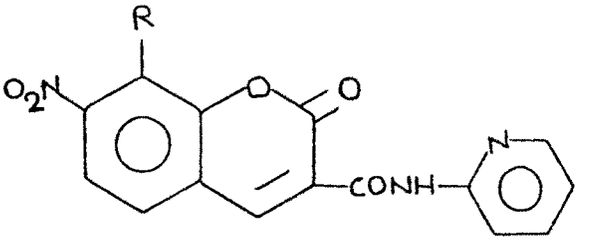
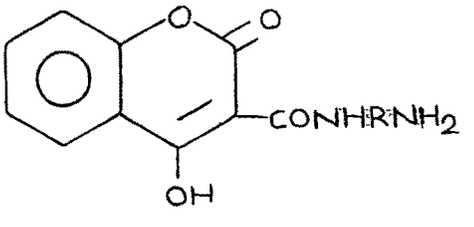


(16)

The bactericidal and fungicidal activities of 3-[(amino-alkyl and aminoaryl)carbamoyl]-4-hydroxycoumarin (17) was reported by McIntyre Johns et al.[13,14] They synthesised 3-(alkoxy-phenyl carbamoyl)-4-hydroxycoumarin (18) from 4-hydroxy-coumarin and phenylisocyanate.



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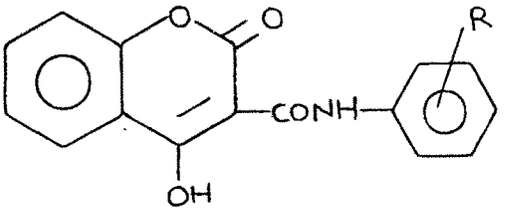
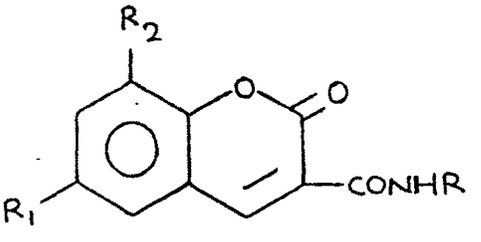


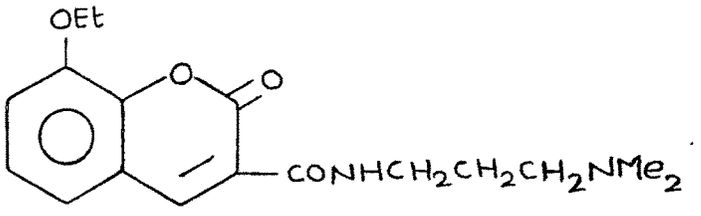
(18)

Recently Mamta Agrawal, S.B. Bansal and O.P. Singhal[15] synthesised coumarin carboxamides (19) by condensing malon-o-phenetidic, 3-chloro-2-methyl, 3-chloro-2-methoxy anilic acids and thiazole-2-malonamic acid with salicylaldehyde and substituted salicylaldehyde. They reported that the compound 6-bromo-coumarin-3-phenetidine was active against the bacteria B. Subtilis and the fungi T. mentagrophytes, A. niger ; 6-chlorocoumarin-3-carboxy (3-chloro-2-methyl)-anilide was active against bacteria S. aureus, B. Subtilis and fungi A. niger ; 6,8-dibromocoumarin-3-carboxy-(3-chloro-2-methoxy) anilide was active against bacteria V. Comma and fungi T. rubram.



(19)

Smidrkal Jan and Hedrlin Ivo[16] prepared 8-ethoxycoumarin-3-carboxamide (20) from 8-ethoxycoumarin-3-carboxylate and $H_2NCH_2CH_2CH_2NMe_2$ which was potentially effective components of creams protective against U.V. radiation.



(20)

Present Work

The literature survey also revealed that haloacetamides[17,18] possess amoebicidal activity and substituted acetamides[19,20] have local anaesthetic property. With a view to prepare better therapeutically active compounds, the present work has been undertaken.

General Method of Preparation

8-Methoxycoumarin-3-carboxanilides and amides (21)

The acid chloride was prepared by treating 8-methoxy-coumarin-3-carboxylic acid with thionyl chloride. This acid chloride was then condensed with various amines to obtain 8-methoxycoumarin-3-carboxanilides and amides.

General discussion of IR spectra

The IR spectra shows characteristic absorption band

of N-H stretching at 3400 (broad), 1720 of >C=O of coumarin and 1600 (aromatic C=C ring stretch) cm^{-1}. It also exhibited an amide I band at 1670-1650 cm^{-1} and amide II band at 1560-1540 cm^{-1}. In case of tertiary amide >C=O absorption was observed at 1680-1630 cm^{-1}.[21] It also exhibited C-O-C absorption bands at 1280, 1100 cm^{-1}.

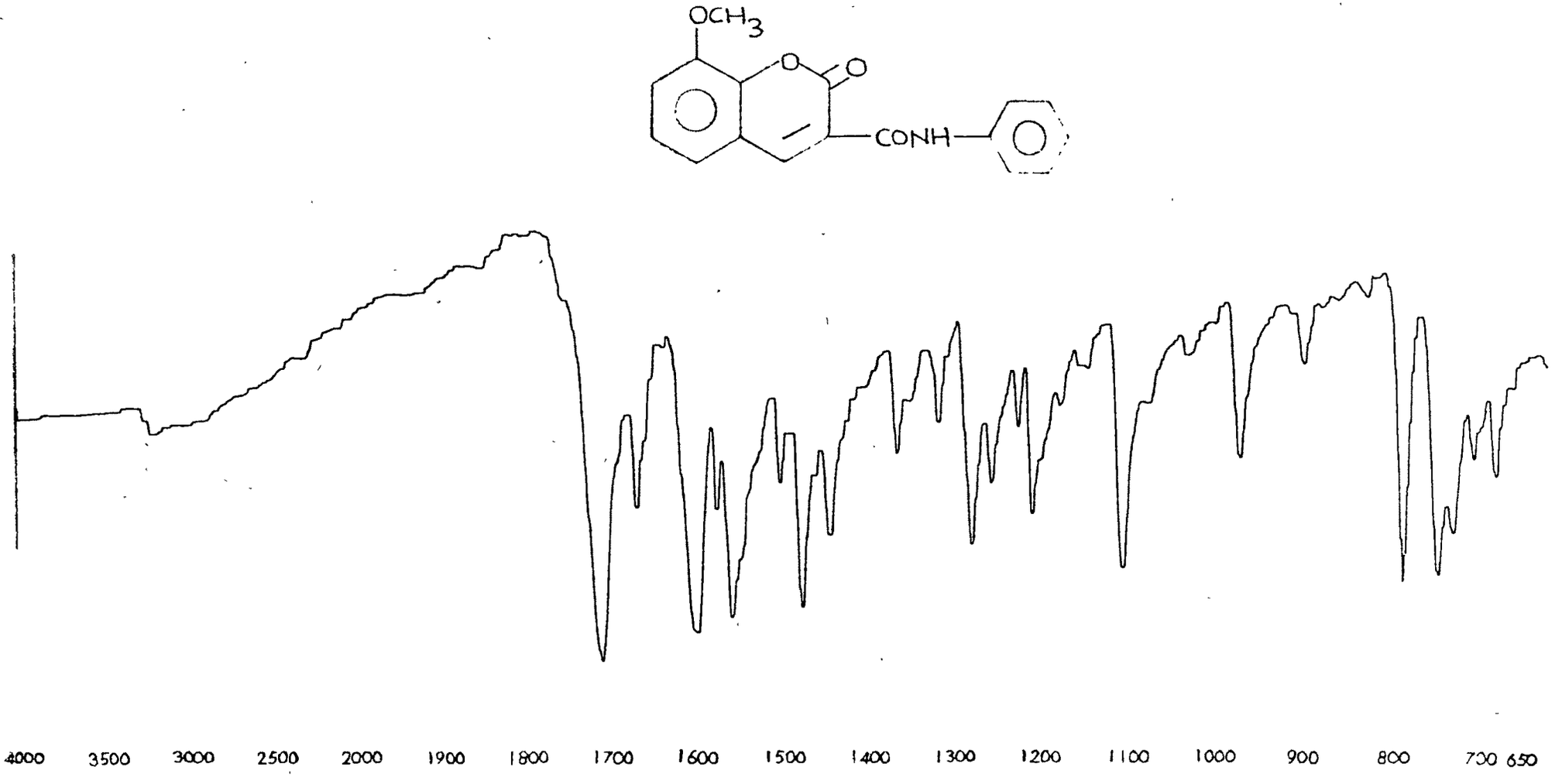
The method of synthesis and spectral data of some individual compounds have been described here to further support the structure assigned to the compounds.

8-Methoxycoumarin-3-carboxanilide (22, Table-I, 1)

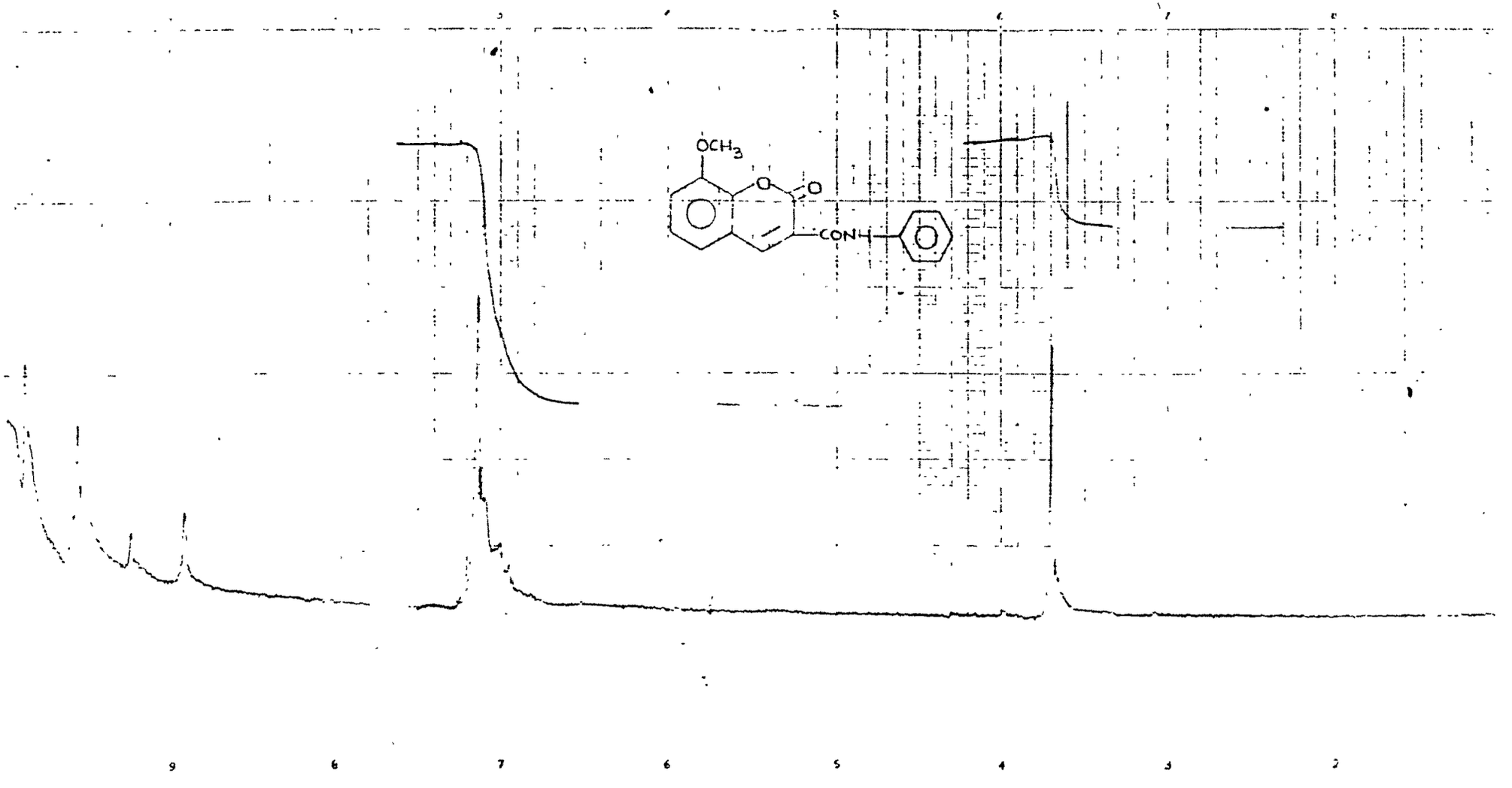
8-Methoxycoumarin-3-carbonylchloride was reacted with aniline to obtain 8-methoxycoumarin-3-carboxanilide. The structure of the compound was proved by following spectral data.

The IR (KBr) spectrum exhibited bands at 3250 (broad), 1710, 1670, 1600, 1280, 1110 cm^{-1} (Fig. 1).

The NMR spectrum (CF_3COOH) showed following signals : δ 3.6 , singlet, protons of group OCH_3 attached to coumarin nucleus at C-8 position ; δ 6.8-7.1, multiplet, aromatic protons ; δ 8.86, singlet, proton at C-4 position of coumarin ring. (Fig. 2).



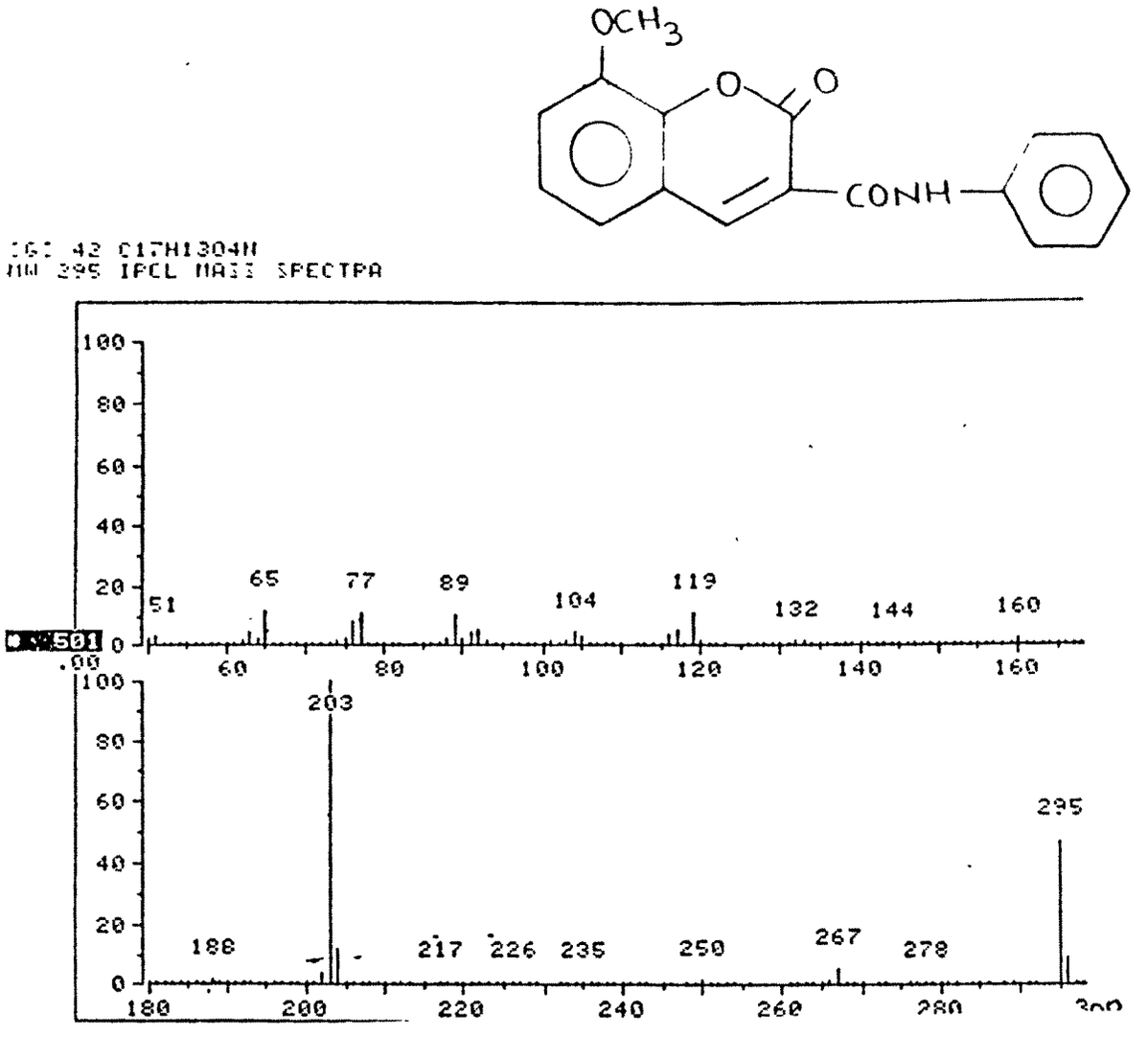
(Fig-1): 22, Table-I, 1



(Fig - 2): 22, Table - I, 1

(0.1 ppm off set)

8268



(Fig- 3): 22, Table- I, 1

The mass spectrum showed signals at m/e : 295 (M^+ ion, 50%), 203 (base peak, 100%), 119 (15%), 89 (15%), 77 (15%), 65 (15%). (Fig. 3).

8-Methoxycoumarin-3-carboxy (N-phenyl) anilide (23, Table-I 20)

The above compound was obtained by condensing 8-methoxy-coumarin-3-carbonylchloride and diphenylamine.

The IR (KBr) spectrum showed bands at 1725, 1640, 1605, 1280 and 1100 cm^{-1} . (Fig. 4).

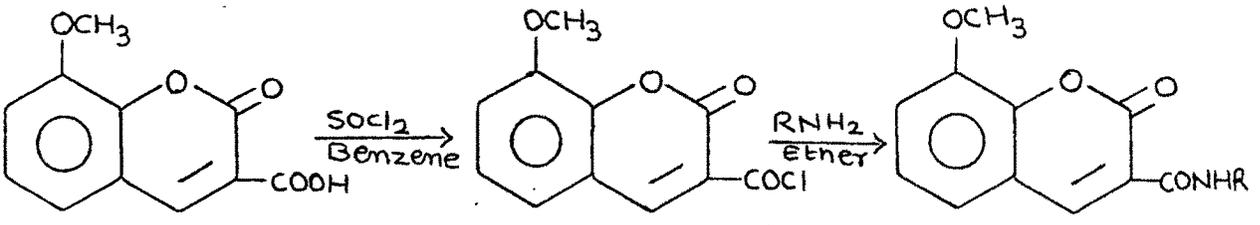
In NMR spectrum, following signals were observed (CF_3COOH) : δ 3.6, singlet, protons of group OCH_3 attached to C-8 position of coumarin ring ; the aromatic protons appeared as multiplet in the region δ 6.8 - 7.1 ; the proton at C-4 position appeared as singlet at δ 7.99 (Fig. 5).

Other carboxanilides and amides were prepared in a similar way (Table-1).

Antifungal and Antibacterial activity

Some selected members of the series were tested for their antifungal and antibacterial activity. Some anilides were found to be active against fungi. None of the compounds

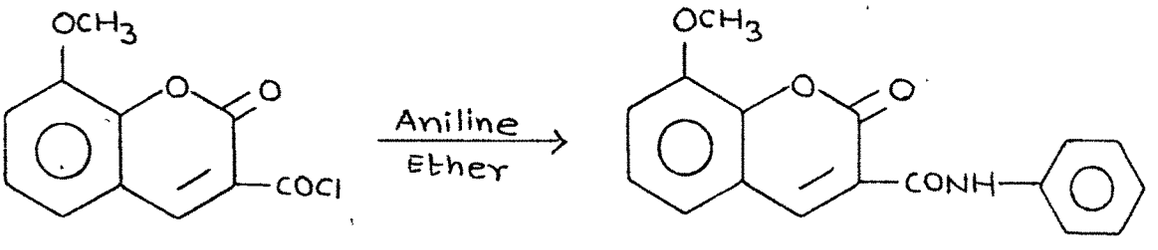
Scheme:1[36]



(21)

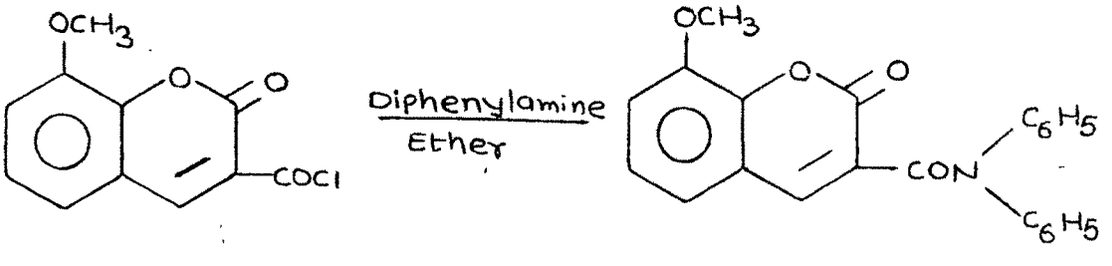
Table-I, 1-24

Scheme:2

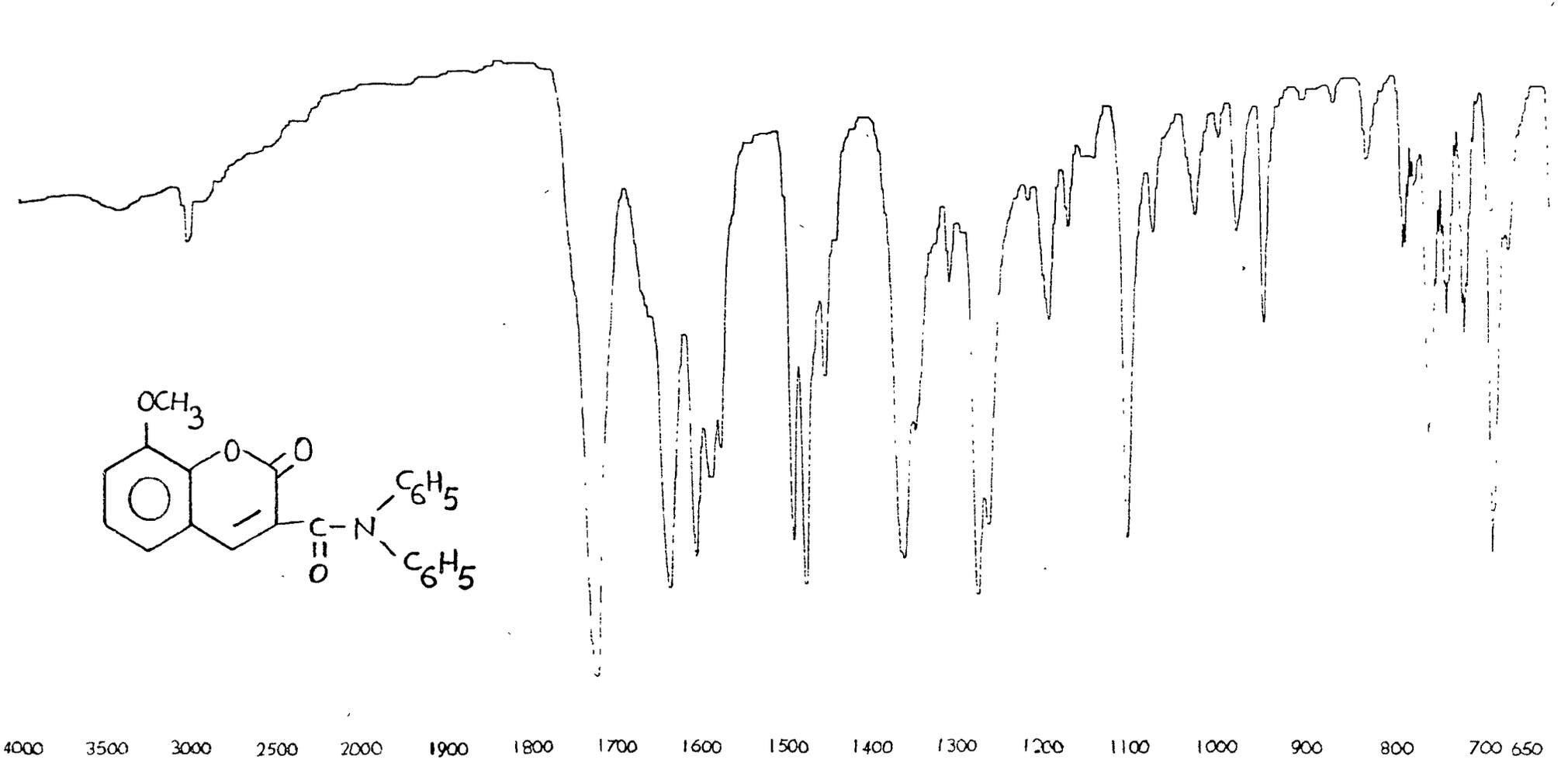


(22)

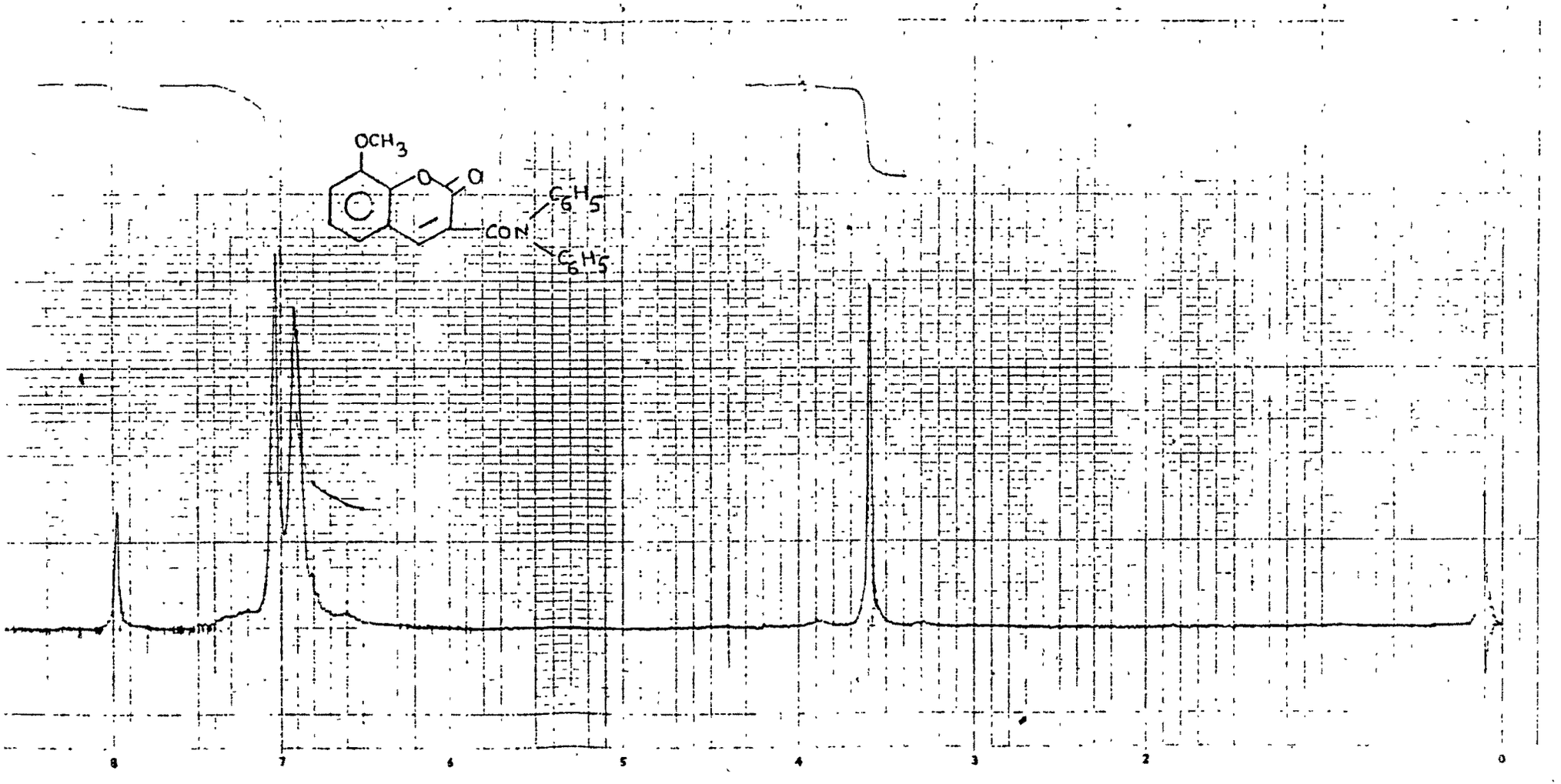
Scheme:3



(23)



(Fig - 4) : 23, Table- I, 20



(Fig - 5): 23, Table - I, 20

was found to have antibacterial activity. Screening report is exhibited and described in Part II of this chapter.

EXPERIMENTAL

EXPERIMENTAL

All melting points are uncorrected. Microanalysis of compounds were performed on a Coleman instrument, IR spectra (KBr) were taken on a Shimadzu 408 spectrophotometer, NMR spectra were recorded on a Perkin-Elmer R-32 Spectrometer, using TMS as the internal standard. Mass spectra were recorded on a GCMS Model Hewlett Packard 5985 analysed at 70 ev.

8-Methoxycoumarin-3-carboxanilide (22, Table-1 1)

A mixture of 8-methoxycoumarin-3-carbonyl chloride (0.01 mole) and aniline (0.01 mole) was stirred in dry ether (30 ml) at room temperature for 3 hrs. The resulting product was filtered and crystallised from chloroform, M.p. 232°C, yield 80%.

Analysis : Found : C, 69.39 ; H, 4.31 ; N, 4.34%

$C_{17}H_{13}O_4N$: requires : C, 69.15 ; H, 4.41 ; N, 4.75%

8-Methoxycoumarin-3-carboxy-(N-phenyl) anilide (23, Table-I,20)

8-Methoxycoumarin-3-carbonylchloride (0.01 mole) and diphenylamine (0.01 mole) were stirred in dry ether (30 ml) at room temperature for 3 hrs. The separated product was crystallised from chloroform, M.p. 215°C, yield 73%.

Analysis : Found : C, 74.42 ; H, 4.91 ; N, 3.23%

$C_{23}H_{17}O_4N$: requires : C, 74.39 ; H, 4.58 ; N, 3.77%

TABLE - I : ANALYTICAL AND PHYSICAL DATA OF 8-METHOXYCOUMARIN-3-CARBOXANILIDES AND AMIDES

Sr. No.	R	R_1	M.P.* °C	Yield %	Molecular Formula	Analysis % Found (calcd) C	H	N
1	C_6H_4-	H	232[c]	80	$C_{17}H_{13}O_4N$	69.39 (69.15)	4.31 (4.41)	4.34 (4.75)
2	o-Cl-C_6H_4-	H	255[b]	85	$C_{17}H_{12}O_4NCl$	62.16 (62.0)	3.79 (3.65)	3.79 (4.26)
3	p-Cl-C_6H_4-	H	197[b]	82	$C_{17}H_{12}O_4NCl$	61.92 (62.0)	3.51 (3.65)	3.96 (4.26)
4	p-Br-C_6H_4-	H	259[c]	75	$C_{17}H_{12}O_4NBr$	54.83 (54.55)	3.66 (3.25)	3.25 (3.74)
5	p-NO_2-C_6H_4-	H	343[dm]	95	$C_{17}H_{12}O_6N_2$	60.10 (60.0)	3.41 (3.53)	7.85 (8.24)
6	m-NO_2-C_6H_4-	H	310[dm]	76	$C_{17}H_{12}O_6N_2$	60.5 (60.0)	3.12 (3.53)	7.92 (8.24)
7	p-CH_3-C_6H_4-	H	235[c]	82	$C_{18}H_{15}O_4N$	70.30 (69.90)	4.86 (4.85)	4.28 (4.53)

cont.....

TABLE-I cont....

8	m-CH_3-C_6H_4-	H	246[c]	85	$C_{18}H_{15}O_4N$	70.31 (69.90)	4.89 (4.85)	4.92 (5.53)
9	p-OCH_3-C_6H_4-	H	230[c]	73	$C_{18}H_{15}O_5N$	66.70 (66.46)	4.42 (4.62)	4.50 (4.31)
10	p-$COOC_2H_5$-C_6H_4-	H	241[c]	86	$C_{20}H_{17}O_6N$	65.88 (65.39)	4.25 (4.63)	3.72 (3.81)
11	o-NH_2-C_6H_4-	H	228[c]	81	$C_{17}H_{14}O_4N_2$	65.92 (65.81)	4.99 (4.52)	8.72 (9.03)
12	o-NH_2-C_6H_4-	Br	295[c](d)	75	$C_{17}H_{13}O_4N_2Br$	52.11 (52.44)	2.99 (3.34)	7.0 (7.19)
13	p-Br-C_6H_4-	Br	265[b+a]	65	$C_{17}H_{11}O_4NBr_2$	45.40 (45.03)	2.83 (2.43)	2.61 (3.09)
14	p-$COCH_3$-C_6H_4-	H	280[d+w]	80	$C_{19}H_{15}O_5N$	68.10 (67.66)	4.35 (4.45)	3.76 (4.15)
15	m-$COCH_3$-C_6H_4-	H	232[d+w]	80	$C_{19}H_{15}O_5N$	68.08 (67.66)	4.85 (4.45)	3.73 (4.15)
16	α-naphthylamine	H	239[c]	79	$C_{21}H_{15}O_4N$	73.40 (73.04)	4.21 (4.35)	3.76 (4.06)
17	β-naphthylamine	H	246[c]	80	$C_{21}H_{15}O_4N$	73.51 (73.04)	4.48 (4.35)	3.97 (4.06)

cont....

TABLE-I cont....

18	Cyclohexylamine	H	160[c]	74	$C_{17}H_{18}O_4N$	68.0 (68.0)	5.94 (6.0)	4.42 (4.64)
19	4-phenylthiazol-2-	H	294[c]	62	$C_{20}H_{14}O_4NS$	63.87 (63.49)	3.92 (3.70)	7.42 (7.41)
20	C_6H_5-	C_6H_5-	215[c]	73	$C_{23}H_{17}O_4N$	74.42 (74.39)	4.91 (4.58)	3.23 (3.77)
21	C_2H_5-	C_6H_5-	193[c]	75	$C_{19}H_{17}O_4N$	70.10 (70.59)	5.49 (5.26)	3.84 (4.33)
22	C_2H_5-	C_2H_5-	120[w]	73	$C_{15}H_{17}O_4N$	65.98 (65.45)	6.59 (6.18)	4.93 (5.09)
23	Morpholine	-	189[c]	69	$C_{15}H_{15}O_5N$	61.86 (62.28)	5.0 (5.19)	4.51 (4.84)
24	N-phenyl-piperazine	-	162[a+b]	91	$C_{21}H_{20}N_2O_4$	69.0 (69.23)	5.46 (5.49)	7.37 (7.69)

---

* Crystallisation solvent : a = alcohol,
b = benzene,
c = chloroform
d = DMF, dm = DMSO, w = water

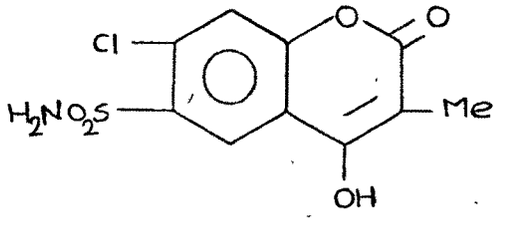
(ii) Synthesis of Sulfonamides of Coumarin Derivatives and Screening them for their Antibacterial activity

INTRODUCTION

It is well-known that sulfanilamide and certain related substituted amides are of considerable medical importance as the Sulfa drugs. The antibacterial activity of these drugs stems from a rather simple fact ; enzymes in the bacteria confuse it for p-amino benzoic acid, which is an essential metabolite. In what is known as metabolite antagonism, the sulfanilamide competes with p-aminobenzoic acid for reactive sites on the enzymes ; deprived of the essential metabolite, the organism fails to reproduce and dies.

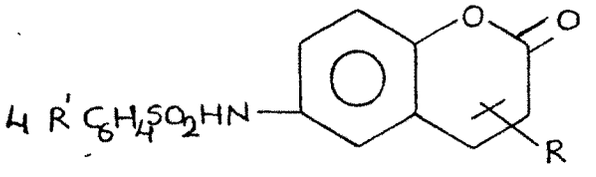
Some recent synthesis of sulfonamido derivatives of coumarin derivatives have been briefly reviewed here.

Robert F. Meyer[22] synthesised 3-allyl-4-hydroxy-7-chloro-coumarin-6-sulfonamides (24) by condensing 6-sulfonylchloride-coumarin with ammonia. These compounds possessed diuretic activity.



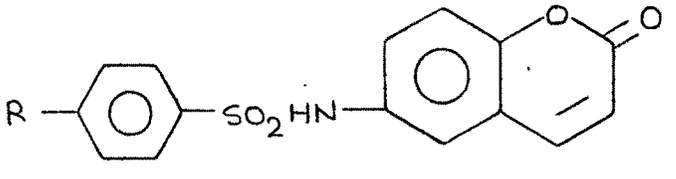
(24)

Haruo Kitagawa and Riichiro Iwaki[23] prepared substituted 6-(p-tolylsulfonamido) coumarin, 6-(p-acetamidosulfonamido) coumarin and 6-(p-amino sulfonamido) coumarin (25) from 6-aminocoumarin and substituted sulfonylchloride. They reported that 6-(p-acetamidosulfonamido) coumarin derivatives possessed greater tuberculostatic activity.



(25)

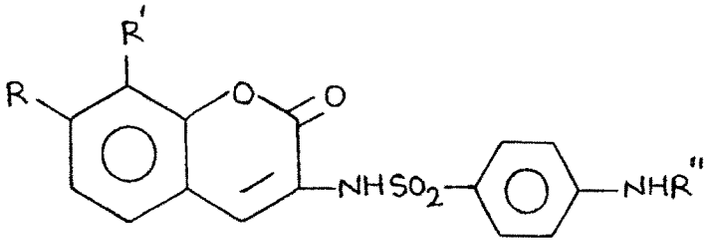
L. Reppel and W. Schmollack[24] had also reported synthesis of Sulfanilamidocoumarin (26) from 3-amino, 6-amino, 8-amino, 3-amino-6-nitro, 3-amino-8-nitro coumarin and P-Ac-NH-$C_6H_4SO_2Cl$.



(26)

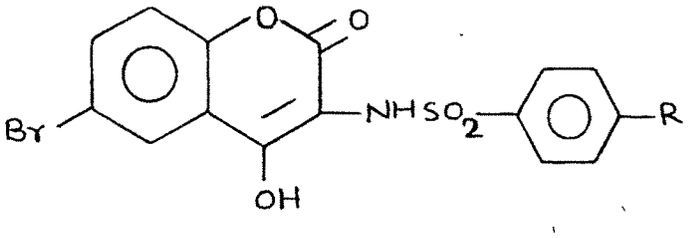
3-(p-acetamidosulfonamido)-7-hydroxy coumarin (27) (R'=H) was prepared by Chakravarti and R. Das,[25] by condensing

p-acetamido-benzenesulfonylchloride with 3-amino-7-hydroxy-coumarin. They also prepared 8-methoxy-3-(p-acetamidosulfonamido) coumarin.



(27)

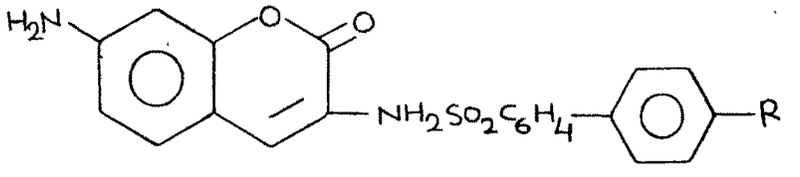
M. Dazelic and coworkers[26] synthesised 3-sulfonamido-4-hydroxy-6-bromocoumarin (28) by refluxing 3-amino-4-hydroxy-6-bromocoumarin with p-acetamidobenzene sulfonylchloride followed by removal of acetyl group.



(28)

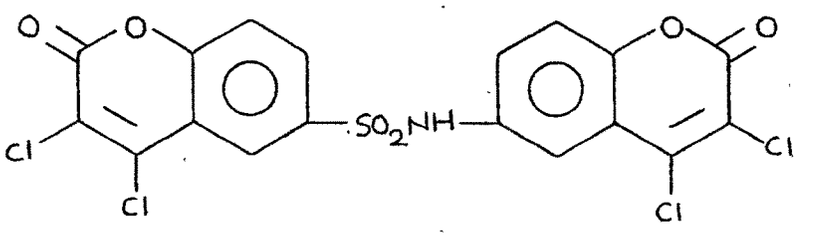
Ichikawa Masataka and Ichibagase Hisashi[27,28] condensed 3,7-diamino-4-hydroxy coumarin with p-substituted benzene sulfonyl chloride to obtain 7-amino-4-hydroxy-3-sulfonamido-

coumarin (29) which was active against Mycobacterium tuberculosis in vitro with MIC of 6.3 μg/ml. They observed that activity was not affected by acetylation of amino group of the sulfanilamide but was greatly reduced by acetylation of amine group in the 7-position of the coumarin ring.



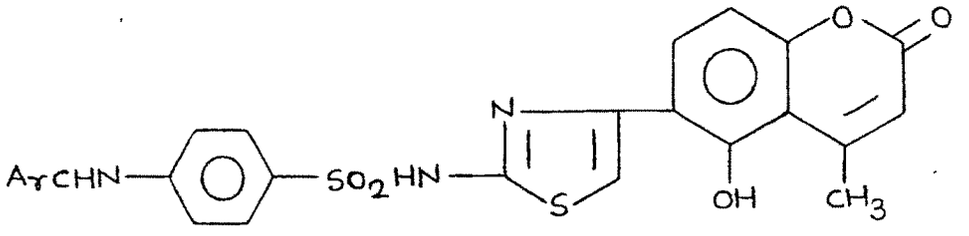
(29)

Bachman Gerald [29] synthesised various sulfonamidocoumarin (30) by condensing aniline or aminocoumarin with a halogenated coumarin sulfonylchloride. Thus, they prepared 6-(3,4-dichlorophenyl sulfonamido)3,4-dichlorocoumarin and other halo derivatives. These compounds were active against gram positive bacteria.



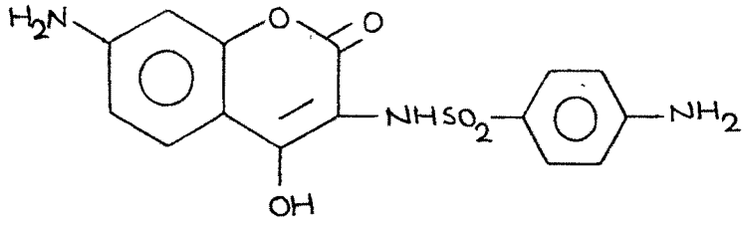
(30)

The 4-methyl-5-hydroxycoumarin derivative of 2-(N^4-acetylsulfanilamido) thiazole (31) were prepared by K.A. Thaker and N.R. Manjaramkar.[30] Some of these compounds inhibited the growth of fungi and inhibited mustard seed germination.



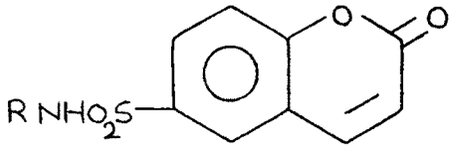
(31)

Daiichi Seiyaky Co Ltd.[31] synthesised 4-hydroxy-7-amino-3-sulfanilamidocoumarin (32) which was useful as a bactericides.

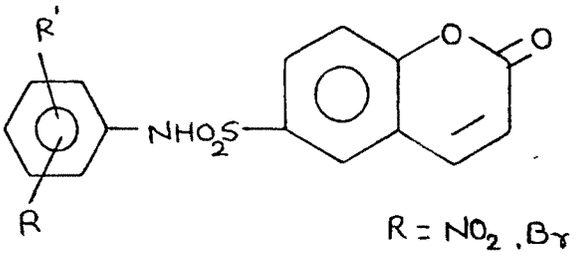


(32)

A.M. Islam and coworkers[32] prepared coumarin sulfonamides (33) by condensing coumarin-6-sulfonylchloride with primary, aliphatic, aromatic and secondary aliphatic amines. They also prepared bromo and nitro-coumarin sulfonamido derivatives (34). Some of the compounds were found to be active against S. aureus.

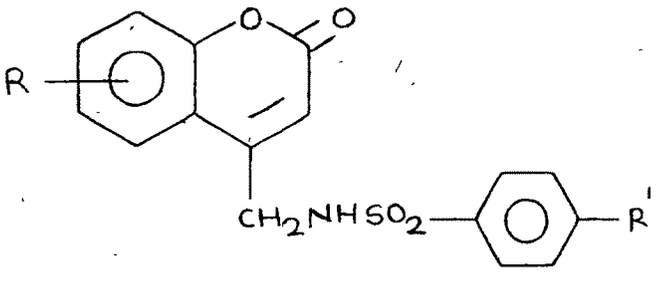


(33)



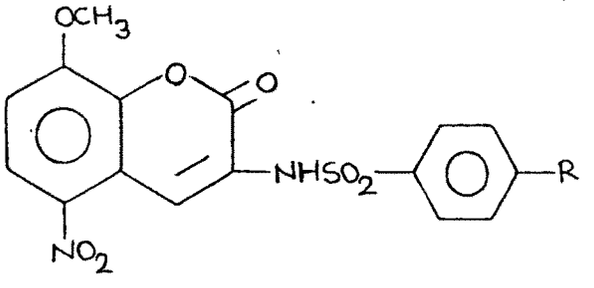
(34)

Hanmantgad Shrikant et al.[33] synthesised number of substituted 4-sulfonamido methyl coumarins (35) which were active against S. auereus and E. Coli.

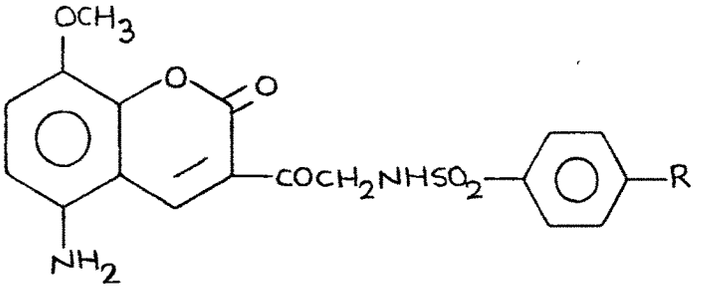


(35)

Sulfanilamido derivatives of 3-amino-5-nitro-8-methoxy-(36), 3-acetylamino-5-amino-8-methoxy-(37), 3-amino-8-hydroxy- and 3-amino-7,8-dihydroxy coumarin were prepared by Antonello Cipriano.[34] They tested their antibacterial activity.



(36)



(37)

Recently Cremlyn Richard and Clowes Sally[35] prepared sulfonyl coumarin derivatives from 6-(chlorosulfonyl)coumarin and various amines. Some of them showed fungicidal activity.

Present Work

References quoted in the earlier paragraphs reveal that introduction of $-SO_2NH-$ group in the substrate molecule like coumarin derivatives induces biological activities. In search of potent drug, it was therefore thought of interest to prepare sulfonamido derivatives from 8-methoxycoumarin-3-carboxy-(o-amino) anilide and 8-methoxycoumarin-5-(o-amino) anilinomethyl coumarin by condensing them with various p-substituted benzene sulfonyl chloride and to observe if the products could display any antibacterial activity.

General Method of Preparation

8-Methoxycoumarin-3-carboxy-[o-(p'-substituted benzene sulfonamido)]-anilide (38)

8-Methoxycoumarin-3-carboxy-(o-amino) anilide and p-substituted benzene sulfonyl chloride were reacted to obtain the title compounds. The structure of the compounds were established by elemental and spectral analysis.

The doublet observed in the IR spectra of starting material (o-amino) anilide in the region 3450-3350 cm^{-1} due to free $-NH_2$ along with -NH (CONH) absorption at 3250 cm^{-1}, disappeared and instead all compounds exhibited, broad band in the region 3250 cm^{-1} due to SO_2NH and NH stretching. This supports formation of sulfonamido derivatives.

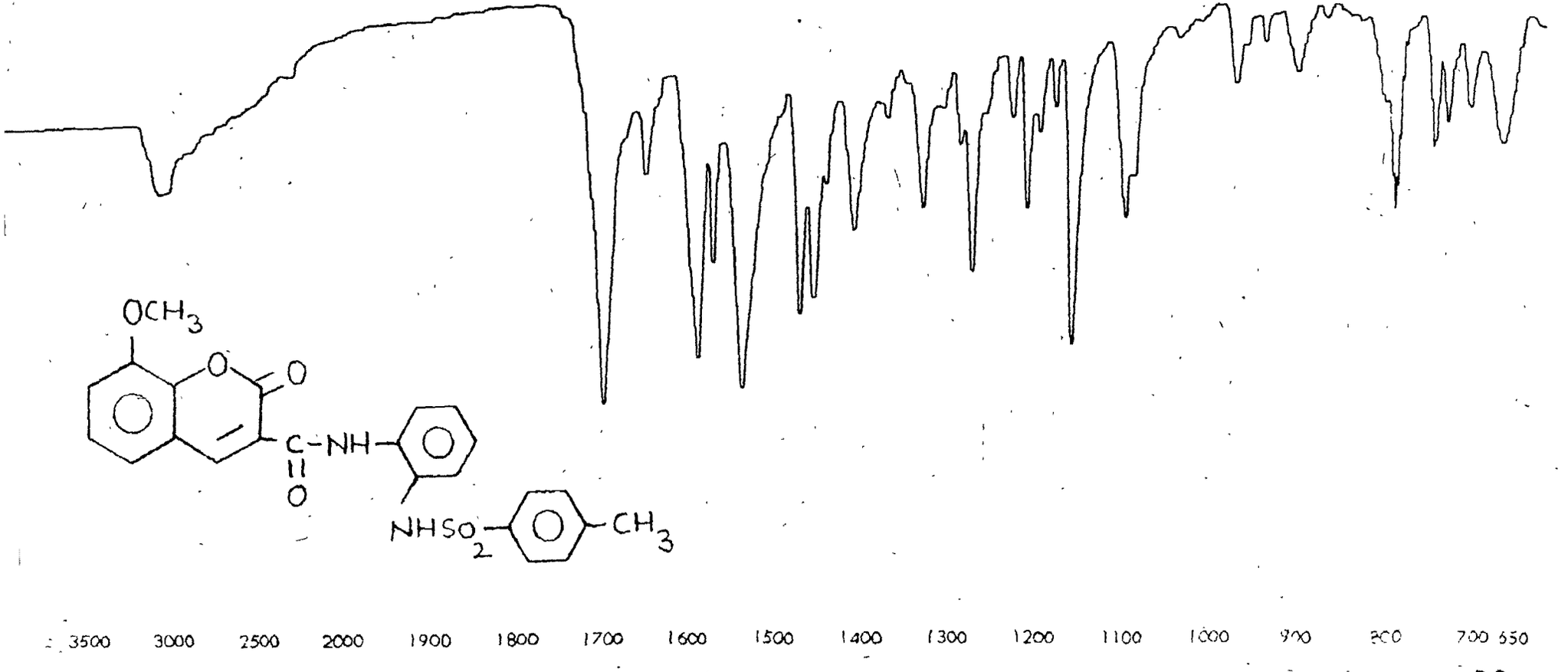
To further support, the structures assigned to sulfonamido derivatives of coumarins, following method of preparation, IR and NMR of sulfonamido compound has been described here.

8-Methoxycoumarin-3-carboxy-[o-(p'-methylbenzene sulfonamido] anilide (38, Table-II, 1)

Condensation of 8-methoxycoumarin3-carboxy (o-amino) anilide and p-methyl benzene sulfonylchloride gave above sulfonamido derivative. The structure of the compound was established by following spectral data.

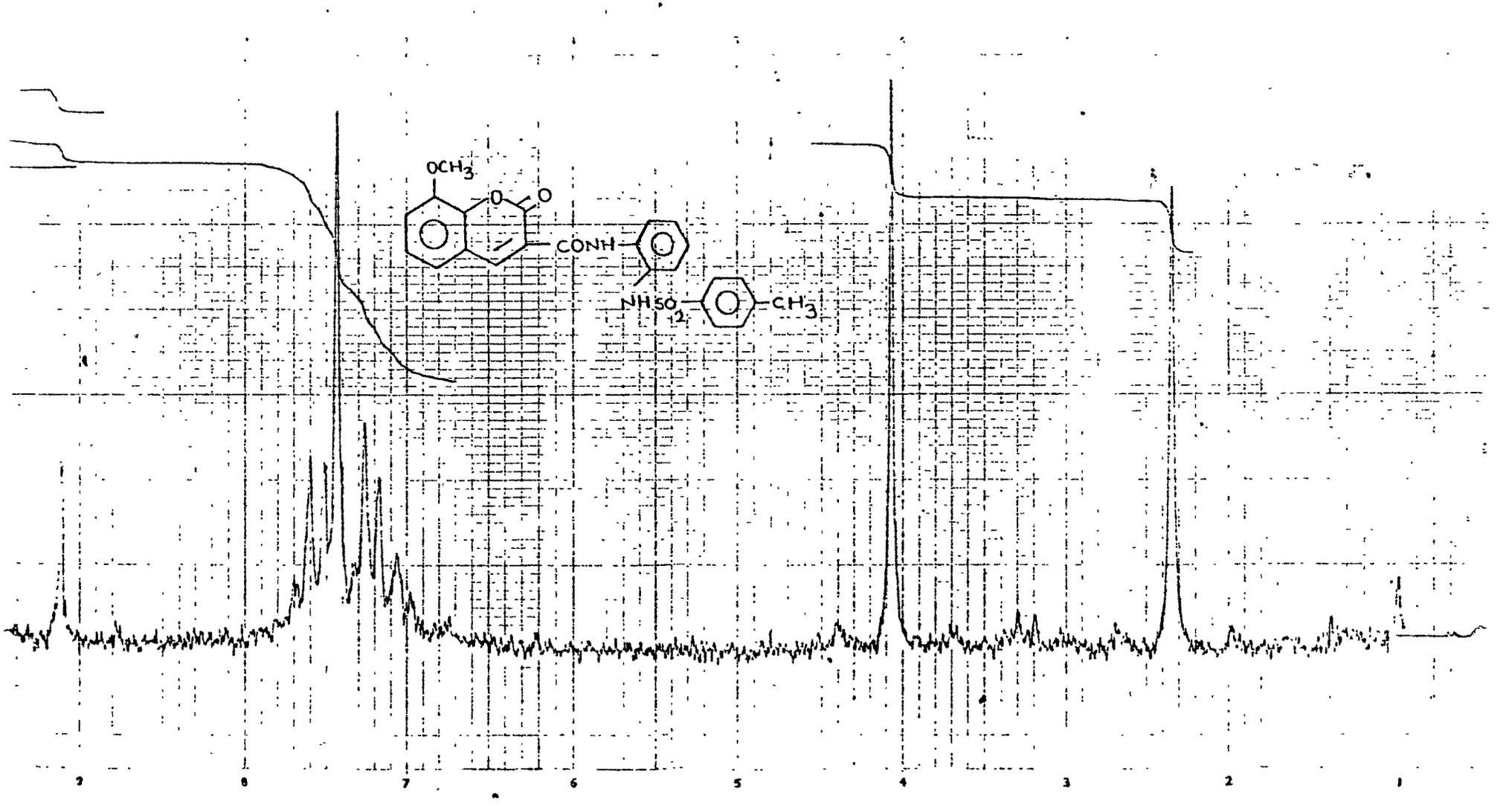
The IR spectra (KBr) exhibited broad band in the region 3200-3000 cm^{-1}, due to SO_2NH and NH stretching, 1700 (lactonic carbonyl of coumarin ring), 1653 (CONH), 1590 (aromatic C=C), 1280 and 1100 (C-O-C) cm^{-1} (Fig. 6).

The NMR spectra (CF_3COOH) exhibited signals at δ 2.13, singlet for three protons of methyl group attached to phenyl ring ; δ 3.87, singlet, OCH_3 protons at C-8 position of coumarin ring ; aromatic protons appeared as multiplet in the region δ 6.9-7.5 ; δ 8.9, singlet, a proton of C-4 position of coumarin ring. (Fig. 7).



882

ig- 6) : 38, Table- II.1



(Fig - 7) : 38, Table- II, 1

(0·2 PPm off set)

General Method of Preparation

8-Methoxy-5-anilinomethyl-[o-(p'-substituted benzene sulfonamido)]coumarin (39)

8-Methoxy-5-(o-amino)-anilinomethylcoumarin and p-substituted benzene sulfonyl chloride were condensed to get above sulfonamido coumarin derivatives. The structures of the compounds were proved by elemental and spectral analysis. The IR spectra of all compounds exhibited two sharp bands around 3400 and 3250 cm^{-1} due to $-SO_2NH-$ and -NH stretching. For additional confirmation of structure following spectral data of sulfonamido compound has been displayed here.

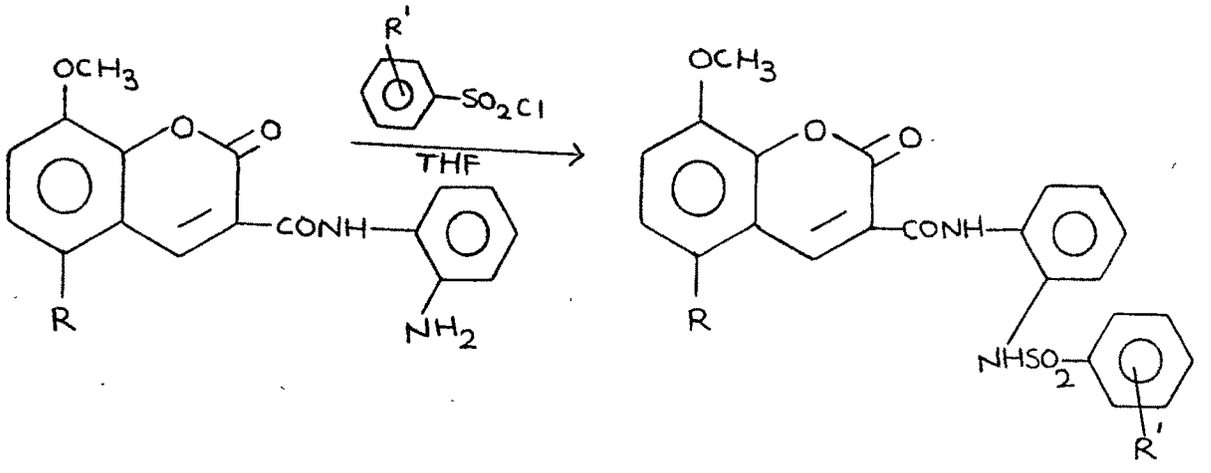
8-Methoxy-5-anilinomethyl [o-(p'-bromo benzene sulfonamido)] coumarin (39, Table-III, 5)

A mixture of 8-methoxy-5-(o-amino)-anilino methylcoumarin and p-bromo benzene sulfonyl chloride was reacted to furnish above sulfonamidocoumarin.

The IR (KBr) spectra showed bands at 3400, 3250, 1710, 1605, 1290 and 1095 cm^{-1} (Fig. 8).

The NMR spectra exhibited signals (CF_3COOH) at δ 3.78, singlet, $OC\underline{H}_3$ protons ; δ 4.92, singlet, $-C\underline{H}_2NH-$ protons of C-5 position ; doublet of C-3 proton appears to overlap on aromatic protons at δ 6.3 which are found as multiplet in the region δ 6.3-8.0. C-4 proton appeared as doublet at δ 7.95 (Fig. 9).

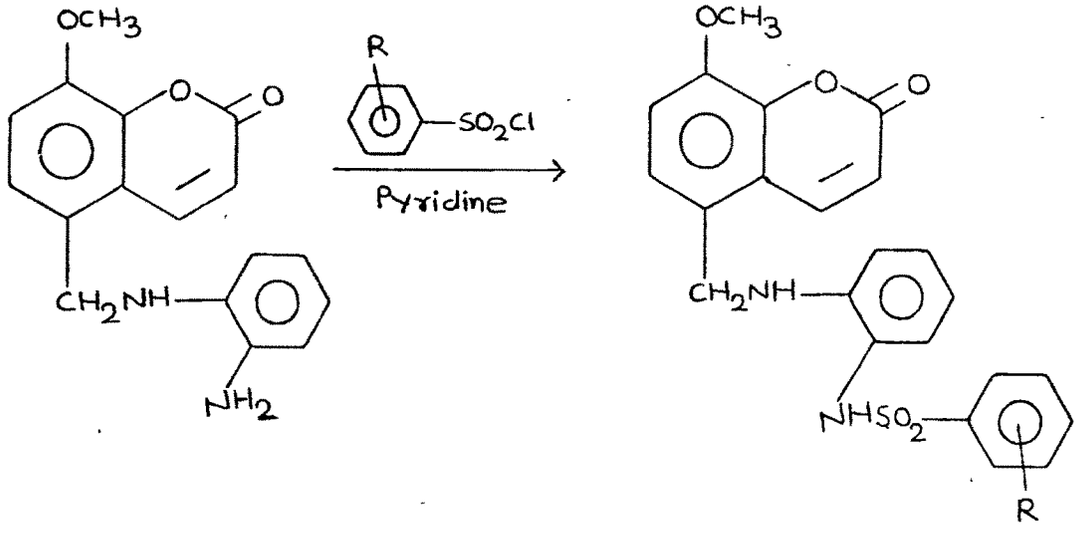
Scheme: 4



(38)

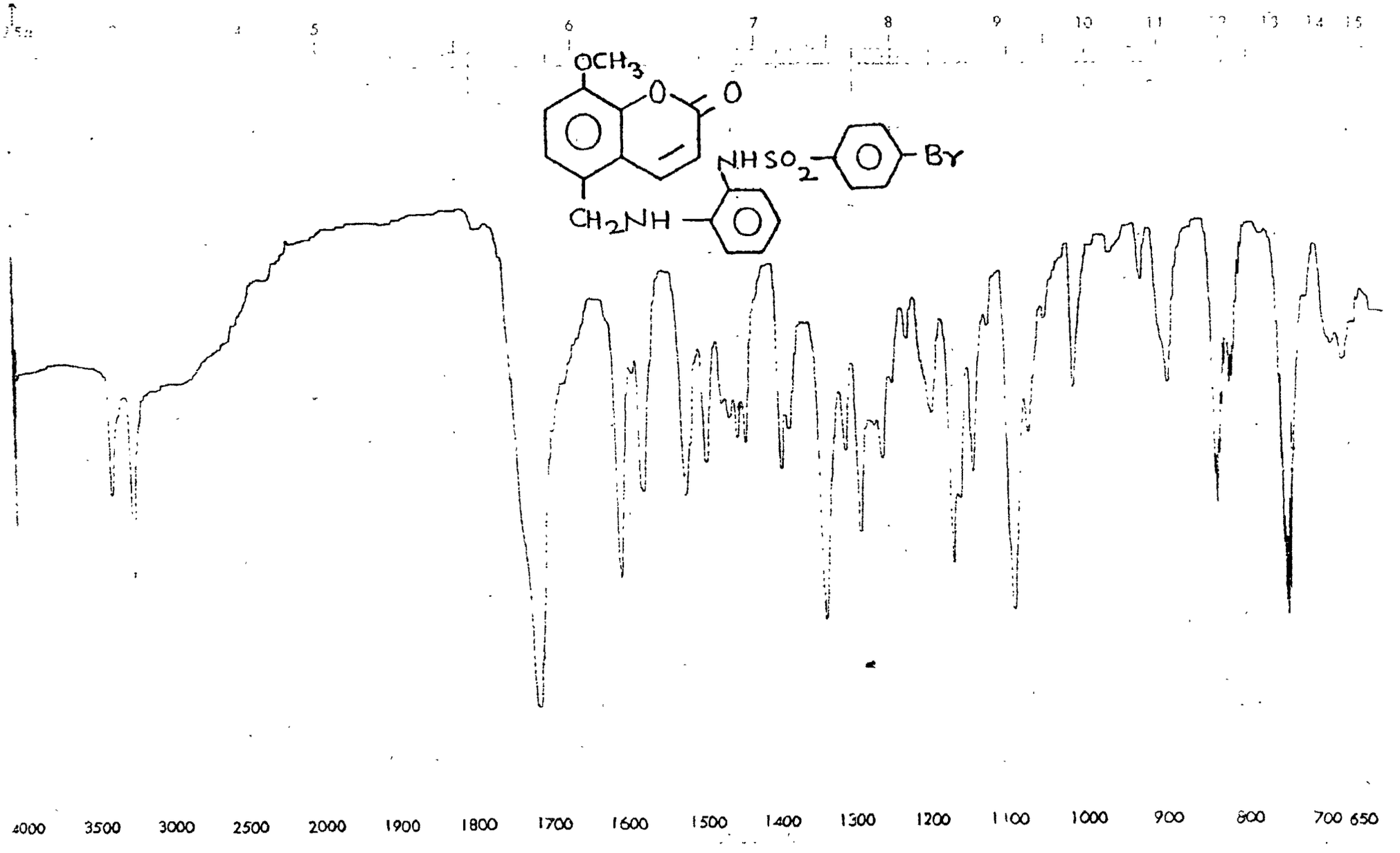
Table - II, 1-10

Scheme: 5

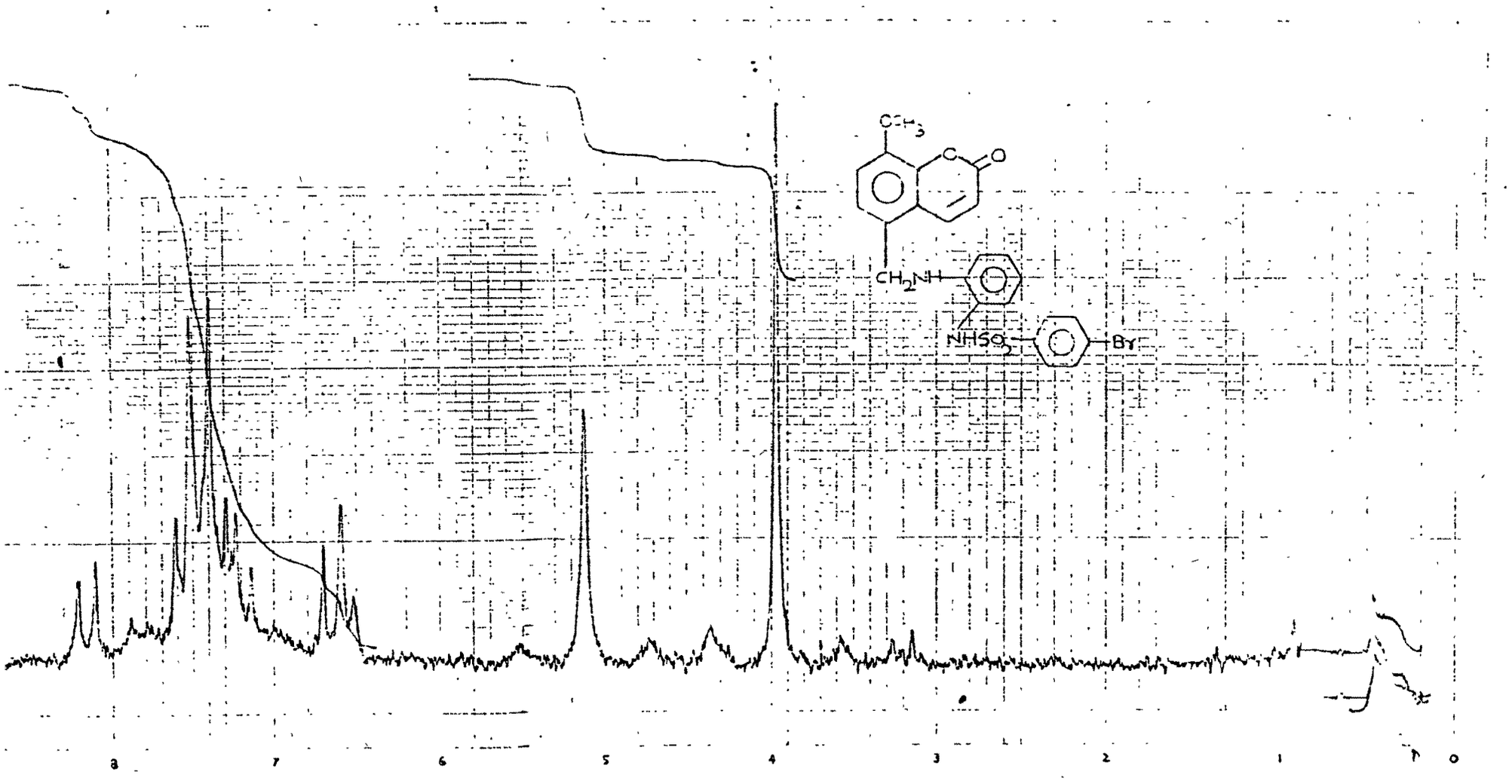


(39)

Table - III, 1-5



(Fig-8): 39, Table-III,5



(Fig- 9): 39, Table- III,5

(0.2 PPm off set)

EXPERIMENTAL

8-Methoxycoumarin-3-carboxy-[o-(p'-methyl benzene sulfonamido)] anilide (38, Table-II, 1)

A mixture of 8-methoxycoumarin-3-carboxy-(o-amino) anilide (0.01 mole) and p-methyl benzene sulfonyl chloride (0.01 mole) was refluxed in tetrahydrofuran (5 ml) on an oil bath at 120°C for 3 hrs. The product obtained on cooling, was filtered and crystallised from alcohol-DMF mixture, M.p. 295°C, yield 65%.

Analysis : Found : C, 62.50 H, 3.85 N, 5.83%

$C_{24}H_{20}O_6N_2S$: requires : C, 62.07 H, 4.31 N, 6.03%

8-Methoxy-5-anilinomethyl-[o-(p'-bromo benzene sulfonamido)] coumarin (39, Table-III, 5)

8-Methoxy-5-(o-amino)anilinomethyl coumarin (0.01 ml) and p-bromo benzene sulfonylchloride (0.01 mole) were heated in pyridine (5.0 ml) on an oil bath at 120°C for 3 hrs. The solid obtained on cooling was treated with dilute hydrochloric acid and then crystallised from alcohol-DMF mixture, M.p. 224°C, yield 75%.

Analysis : Found : C, 54.00 ; H, 3.92 ; N, 5.41%

$C_{23}H_{19}O_5N_2Brs$: requires : C, 53.59 ; H, 3.69 ; N, 5.44%

TABLE-II : ANALYTICAL AND PHYSICAL DATA OF 8-METHOXYCOUMARIN-3-CARBOXY-[o-(p'-SUBSTITUTED BENZENE SULFONAMIDO)] ANILIDE(38)

Sr. No.	R	R_1	M.P.* °C	Yield %	Molecular formula	Analysis % Found/(calcd) C	H	N
1.	H	$-CH_3$	295^{a+d}	65	$C_{24}H_{20}O_6N_2S$	62.50 (62.07)	3.85 (4.31)	5.83 (6.03)
2	H	$-OCH_3$	310^{d+w}	60	$C_{24}H_{20}O_7N_2S$	60.41 (60.00)	4.31 (4.17)	5.61 (5.83)
3	H	$-NHCOCH_3$	$280^{a+d}_{(d)}$	70	$C_{25}H_{21}O_7N_3S$	58.63 (59.17)	4.55 (4.14)	7.93 (8.28)
4	H	-Cl	295	80	$C_{23}H_{17}O_6N_2SCl$	56.8 (57.02)	3.10 (3.51)	5.31 (5.79)
5	H	-Br	290	85	$C_{23}H_{17}O_6N_2SBr$	51.93 (52.17)	3.33 (3.21)	4.84 (5.29)
6	Br	$-CH_3$	282	81	$C_{24}H_{19}O_6N_2SBr$	52.81 (53.04)	3.62 (3.49)	5.02 (5.16)

cont.....

TABLE-II Cont....

7.	Br	$-OCH_3$	261	75	$C_{24}H_{19}O_7O_2SBr$	51.16 (51.52)	3.06 (3.39)	4.7 (5.01)
8.	Br	$-NHCOCH_3$	202^{a+w}	75	$C_{25}H_{20}O_7N_3SBr$	51.0 (51.19)	3.09 (3.41)	7.49 (7.17)
9.	Br	-Cl	$290^{d+w}_{(d)}$	82	$C_{23}H_{16}O_6N_2BrSCl$	48.76 (49.02)	2.41 (2.84)	4.66 (4.97)
10.	Br	-Br	$275^{d+w}_{(d)}$	85	$C_{23}H_{16}O_6N_2SBr_2$	45.03 (45.39)	2.51 (2.63)	4.2 (4.61)

---

* Solvent of crystallisation : a = alcohol
d = DMF
w = water

TABLE-III : ANALYTICAL AND PHYSICAL DATA OF 8-METHOXY-5-ANILINOMETHYL-[o-(p'-SUBSTITUTED BENZENE SULFONAMIDO)] COUMARIN (39)

Sr. No.	R	M.P.* °C	Yield %	Molecular Formula	Analysis % Found/(calcd) C	H	N
1	$-CH_3$	197^{a+w}	65	$C_{24}H_{22}O_5N_2S$	63.73 (64.0)	4.52 (4.89)	5.98 (6.22)
2	$-OCH_3$	240^{d}	60	$C_{24}H_{22}O_6N_2S$	61.51 (61.8)	4.50 (4.72)	5.7 (6.01)
3	$-NHCOCH_3$	266^{a+d}	63	$C_{25}H_{23}O_6N_3S$	60.51 (60.85)	4.33 (4.67)	8.1 (8.52)
4	-Cl	224^{a+d}	75	$C_{23}H_{19}O_5N_2ClS$	58.52 (58.72)	4.23 (4.04)	5.66 (5.96)
5	-Br	224^{a+d}	75	$C_{23}H_{19}O_5N_2BrS$	54.0 (53.59)	3.92 (3.69)	5.41 (5.44)

* Solvent of crystallisation : a = alcohol,
d = DMF
w = water

PART – II

TESTING OF ANTIBACTERIAL ACTIVITY OF COMPOUNDS SYNTHESISED IN CHAPTER II TO IV

CHAPTER - IV

PART-II : TESTING OF ANTIBACTERIAL ACTIVITY OF COMPOUNDS SYNTHESISED IN CHAPTER-II TO IV

INTRODUCTION[1-4]

The therapeutics known before the time of Ehrlich were cinchona for malaria, ipeac for amoebic dysentery and mercury for treating syphilis. The diseases of protozoal and spirochaetal origin have been made to respond to synthetic chemotherapeutic agents during the first two decades of 19th Century. The microbiologist and clinical personnel overlooked the possibility that the bacteriostatic compounds would inhibit rapid reproduction of pathogenic bacteria and enable the leucocytes and other defence mechanism of the host to cope with few static invaders.

Paul Ehrlich, the father of chemotherapy used the term chemotherapy to describe the cure of an infectious disease without injury to the host known as chemotherapeutic agents and classified according to diseases and the infections, such as antibacterial, antiprotozoal, antiviral, antineoplastic, antitubercular and antifungal agents.

This part describes methods used for 'invitro' assessment of antibacterial agents. Antibacterial substances and

preparations are classified as disinfectants, antiseptics and chemotherapeutic agents. The term disinfectant is used to eliminate or destroy infection and should be capable of killing a wide range of bacteria. An antiseptic is used to control or eliminate bacterial infection. A chemotherapeutic agent is an antibacterial substance administered systematically for the treatment of infection, may be either bacteriostatic or bacteriocidal in its action, its main function is to prevent the multiplication of infective organism.

Antibacterial agents

They are one type of chemotherapeutic agents used against the bacterial diseases and divided into two types according to their action onbacteria namely bacteriostatic and bactericidal agents. An agent is considered "bacteriostatic" when it inhibits further growth or multiplication of bacteria and classed as "bactericidal" when it kills the bacteria. Antimicrobial agents are the chemotherapeutic substances that destroy or inhibit the growth of micro organisms in the living tissue. Antibiotics are substances produced by living organisms and are sufficiently non-toxic to be used as antimicrobial agents.

CLASSIFICATION OF ANTIBACTERIAL AGENTS

(i) Alcohols and related compounds

Various alcohols and alcohol derivatives have been used as antiseptics, e.g. ethanol and propanol. The antibacterial values of straight chain alcohols increase with an increase in the molecular weight and beyond C_8 the activity begins to fall off. The isomeric alcohols show a drop in activity from primary to secondary to tertiary.

(ii) Acids and their derivatives

Salicylic acid has strong antiseptic and germicidal properties being a carboxylated phenol. The presence of the carboxy group appears to have -Ve effect. Benzoic acid is used externally as an antiseptic and employed in lotions and ointments.

(iii) Iodine containing compounds

Not many iodine containing compounds are widely used as antiseptics in the medicine today. But iodine as a tincture or in aqueous solution with an iodide is still widely used as an antiseptic, virucide, fungicide and amebicide.

(iv) Chlorine containing compounds

The bactericidal properties of hypochlorite was first studied by Robert Koch in 1881. N-Chloro-compounds are represented by amides, imides and amidines in which one or more of the hydrogen atoms attached to nitrogen have been replaced by chlorine, like chloroamine-T, halozone, chlorozidin, etc.

(v) Oxidizing agents

Oxidizing agents are of value as antiseptics depending on the liberation of oxygen, like H_2O_2, other metal peroxides, urea peroxide etc.

(vi) Bacteriostatic dyes

Prior to the advent of the sulfonamides and the antibiotics, the organic dyes have been used extensively as antibacterial agents. Their medical significance was first recognized by Churchman[5] who reported in 1912 on the inhibitory effect of crystal violet on Gram-positive organism. The yellow acridine dyes have been first introduced by Ehrlich for control of trypanosomal infections. Browing[6] in 1913 discovered their antibacterial properties which led to their wide clinical use. The acridines

exert a bactericidal and bacteriostatic action against both Gram-positive and Gram-negative organisms.

(vii) Antibacterial antibiotics

In the twenty years since the discovery of erythromycine, more than fifty antibacterial antibiotics with a common chemical feature - a macrolytic lactone has been described. These macrolides are of great interest because of their antibacterial activity, primarily against Gram positive bacterial and mycoplasma species like methymycin, erythromycin and carbomycin. The streptomycins and neomycine comprise the aminoglycoside antibacteial antibiotics.

(viii) 8-Hydroxyquinoline

8-Hydroxyquinoline or oxime is unique among the isomeric hydroxy quinolines. It alone exhibits antimicrobial activity attributed by its ability to chelate metals.[7]

(ix) Antibacterial metal ions

Metals and their salts other than mercury and silver are less important as practical antibacterial agents. Both organic and inorganic copper salts, used mainly as industrial fungicides and preservatives, are strongly bacteriostatic but lack significant

bactericidal properties. The zinc pyrithione is both highly antibacterial and antifungal. Sprowls and Poc[8] studied the antibacterial activity of a number of metallic salts in solution and found them more effective.

DETOXICATION OF ANTIBACTERIALS

p-Amino benzoic acid is a growth factor for certain microorganisms and competitively inhibits the bacteriostatic action of sulfonamides. The metabolites identified in man are p-aminobenzoylglucuronide : p-amino hippuric acid ; p-acetylaminobenzoylglucuronide ; p-acetylaminohippuric acid and p-acetylaminobenzoic acid. The aromatic nitriles appear to undergo primarily hydroxylation and to a lesser extent, hydrolysis with or without oxidation.

BACTERIA

In 1928, a German Scientist C.E. Ehrenberg used the term 'bacterium'. The bacteria are small microscopic organism with a relatively simple and primitive form of the cellular organisation known as "Procaryotic". The staining reactions of bacteria are of greatest importance in their differentiation and identification. In 1884, Danish physician Gram discovered the stain known as Gram-stain. Staining reaction

has widest application which divided all bacteria into two categories, namely "Gram-(+)ve" and "Gram-(-)ve". The Gram(+)ve bacteria resist decolourisation and remain stained as dark purple colour while Gram(-)ve bacteria are decolourised.

Bacteria can be classified according to their morphological characteristics as lower and higher. The lower bacteria are of generally unicellular structures, never in the form of a mycelium or sheathed filaments, e.g. cocci, bacilli, vibrios, spirille and spirochaetes. The micro-organisms capable of producing disease in animal or human being are known as "Pathogenic". Most of the micro-organisms present on the skin and mucous membranes are non-pathogenic.

CLASSIFICATION OF IMPORTANT ORGANISMS

Class : Schizomycetes

Order	Family	Genus	Species
1	2	3	4
A. Eubacterials	Micrococeaceae	Staphylococcus	Staph. aureus
		Micrococcus	M. tetragenus
		Sarcina	S. lutes
	Lactobacillaceas	Streptococcus	Str.pyogenes
		Peptostreptococcus	Pep.putridis
		Diplococcus	D.pheumoniae
		Lactobacillus	L. acidophilus
	Neisseriaceae	Neisseria	N. gonorrhoeae
			N.meningitidis
			N.catarrhalis
	Corynebacteriaceae	Corynebacterium	C.diphtheriae
		Listeria	E.rhusiopathiae
		Erysipelothrix	L.monocytogenes
	Achromobacteriaceae	Alceligenes	Alc. faecalis
	Enterobacteriaceae	Escherichia	Esch. coli
		Klebsiella	K.pneumoniae, K.aerogenes
		Citrobacter	Cit.Freundii
		Cloaca	Cl. cloacae
		Hafnia	Haf. alvei
		Serretia	Ser.marcescens
		Salmonella	Salm.typhosa
		Shigella	Sh. dysenteriae
		Proteus	Pr. vulgaris

Table cont...

	Brucellaceas	Pasteurella	P.pestis P.psuedotuber-culosis
		Fancisella	F.tularensis
		Brucella	Br.melitensis Br.abortus Br.Suis
		Haemophilus	H.influanzaa H.duoreyi
		Bordetella	Bord.pertussis
		Moraxella	M.lacunata
		Actinoba-cillus	A.mallei, A.lignieresii
	Bacterio-daceae	Bacteroids	Bact.fragilis
		Fusobacterium	F.fusiforms
		Streptobacillus	St.moniliformis
		Sphaerophorus	Sph.necrophorous
	Bacillaceae	Bacillus	B.anthracis B.subtilis
		Clostridium	Cl. tetani, Cl.welchii
B. Pseudo-minadales	Pseudomin-adaceae	Pseudomonas	Ps.aeruginosa
	Spirill-aceae	Vibrio	V.cholerae
		Spirillum	Sp.minus
C. Mycopla-smatales	Mycoplas-mataceae	Mycoplasma	M.pneumoniae M.mycoides
D Actinomy-cetatea	Mycobact-riaceae	Mycobacterium	Myco.tubercu-losis, Myco.laprae

cont...

	Actinomycetaceae	Actinomyces	A.israeli, A.bovis
		Nocardia	N.madurae
	Streptomycetaceae	Streptomyces	Strepto.griseus
E. Spirochaetales	Spirochaetaceae	Spirochaeta	Non.pathogenic
		Saprospirs	
	Treponemataceae	Borrelia	Bor.duttoni, Bor.recuraentis, Bor.vincenti
		Treponema	Tr.pallidum Tr.partenue
		Leptospira	L.icterohaemorrhagies

We have employed five bacterial species namely E.Coli, S.aureus, S.albus, S.typhosa and B.Subtilis for testing the antibacterial activity of these compounds.

EVALUATION OF ANTIBACTERIAL ACTIVITY

Varieties of 'invivo' screening methods has been used to evaluate the antibacterial activity. Testing in mice has become standard, the sensitivity of bacteria to antimicrobial agents is tested by the same methods as in other form of microbiological assey.

Invitro methods

'Invitro' testing is useful for antibacterial spectrum determination of a compound and comparing it with other agent.

Several types of procedure are in use for assaying the potency of antibiotic preparation for therapeutic purpose. These methods have been modified and used for sensitivity test of unknown organisms.

(i) Serial dilution in Broth[9]

Serial dilutions of the drug being assayed are made in uniform amounts of standard broth in culture tubes. These are inoculated with a uniform number of cells to test organism. After incubation, turbidity

(or its absence) is measured by turbidimeter and turbidities (amounts of growth) are compared with a dilution series made in the same way but with antibiotic reference standard of measured potency.

(ii) Streak Assay in agar (loc. cit.)[9]

Graded dilutions of the substance to the tested are placed in a series of petri dishes in which is poured about 10 ml. of melted and cooled agar, contents mixed with drug dilution. After agar has hardened, the plates marked into several sectors, each of which is streaked with different test organism.

(iii) Diffusion tests

Diffusion tests on solid media have been adopted by most of the laboratories wherein the antimicrobial agent is held in a reservoir from which it diffuses through agar medium to form a diffusion gradient to which the microorganisms, growing in or on the agar, are exposed. The size of inhibition zone depends upon the factor that influence the diffusion of the antimicrobial agent as well as the rate of growth of the organism.

(a) Agar strip diffusion test for sensitivity

This is a simple technique which has originally been used by Fleming. A strip of agar is cut from

the centre at a place of suitable culture medium. Appropriate amount of antimicrobial agent is added to molten agar and pipetted into the gutter in the medium and the surface of the agar is inoculated by stroking cultures to be tested.

(b) Replica plate method to show bacteriostatic and bactericidal action[10]

A zone of inhibition of growth around a dish may indicate that the antimicrobial agent is either bactericidal or bacteriostatic. The presence or absence of living organisms within the zones of apparent complete inhibition of growth on diffusion plates have been shown by replica plate method.

(c) Diffusion tests with filter paper-disks for DETERMINING SENSITIVITY[11]

This constitute a simple and reliable technique, impregnating small disk of standard filter paper with given amount of antibiotic placing them on plates of culture medium inoculated with the organism to be tested. After incubation the degree of sensitivity by measuring the easily visible areas of inhibition of growth which has been produced by the diffusion of antibiotic from the disk into the surrounding medium is determined.

DISCUSSION

Certain characteristic features of representative species i.e. one gram +ve and one gram -ve bacterial strains have been briefly described here.

(1) Staphylococcus aureus family : Micrococcaceae

In 1878, Koch observed micrococcus like organisms in pus; Pasteur (1880) cultivated these cocci in liquid media. Ogaston (1881) found it present in pus of acute chronic abscesses and found it pathogenic for mice and guinea pigs.

They are Gram-(+) cocci, ovoid or spheroidel, non-motile, arranged in group of clusters; grow on nutrient agar and produce colonies, which are golden yellow, white or lemon yellow in colour; serobes or faculative anaerobes; biochemical activities and haemolytic power are variable; pathogenic strains produce coagulase, ferment glucose, lactose, mannitol with production of acid, liquefy gelatin and produce pus in the lesion.

Genus : Staphylococcus

Staphylococcus is differentiated from micrococcus,

another genus of the same family, by the ability to utilise glucose, mannitol and pyruvate anaerobically. Staphylocci are found on the skin or mucous membranes of the animal body, especially of the nose and mouth, where they often occur in large numbers even under normal conditions.

Species : Staphylococcus aureus

The individual cells are 0.8 to 0.9 μ in diameter. They are ovoid or spherical, non-motile, noncapsulated, non-sporing, stain with ordinary aniline dyes and Gram-(+)ve, typically arranged in groups. These are aerobbes or facultative anaerobes and grow easilyon nutrient agar. The optimum temperature for the growth is 37°C but the range of temperature varies from 10° to 40°C, optimum pH is 7.4 to 7.6.

(2) Escharichia coli - Family : Enterobacteriaceae

They are Gram (-)ve rods, motile with peritrichate flagella, or non-motile. They do not form spores, and are primarily environmental saprophytes and scavengers, found in the intestinal tract of man or lower animals, hence the family name.

Genus : Escherichia

This genus comprises Escherichia coli and several varients, and is of particular interest since they occur commonly in

the normal intestinal tract of man and animals. Escherichia coli is most distinctively fecal species.

Species : Escherichia coli

Escherichia in 1885 discovered Escherichia coli from the faeces of the new born who showed the organisms in the intestine within 3 days after birth. These are Gram (-)ve rods 2 to 4 μ , commonly seen in coccobacillery form and rarely in filamentous forms. E. coli are generally non-pathogenic and incriminated as pathogens, because sometimes strains have been found to produce septicemia, inflammations of liver gall bladder, appendix, meningitis, pneumonia and other infections.

In vitro testing

Bacteriostatic activity can be determined on solid or liquid, media, each depends on assessing the extent of inhibition of growth. We have adopted " The disk or Cup-plate method" for the sensitivity testing.

The disk method

After the report of the " International Collaborative Study"[12] involved with investigating 'the disk test', the method recommended has been adopted in Sweden. In U.S.A the modified Kirby-Bauer[13] technique has been adopted as an official method by 'Food and Drugs Administration'.

The main stimulus for standardization in U.K. has come from recommendation of use of the controlled single disk method.[14] In this method, nutrient agar of appropriate composition is heavily inoculated with the desired organism all over the surface of the solidified agar or mixed with agar, while still fluid, before pouring the plate. If an antibiotic solution of unknown potency is being assayed, the organism used is stock strain of known sensitivity to standard doses of antibiotic. Measured strengths of the antibiotic solution are applied to the inoculated agar in disks of uniform thickness, or sterile filter paper, are placed on the surface of the agar plate before incubating. The width of the zone indicates, the sensitivity of the organism being tested though the presence or absence of a zone, is of greater significance.

Factors influencing inhibition zone sizes

(i) Ingredients of culture media

Many substances are present in culture media which may affect the zone of inhibition, common ingredients such as peptone, tryptone, yeast extract and agar may vary in their mineral content and many of them may influence the activity of some antibiotics. It is well known that Ca, Mg and Fe affect sensitivity zones produced by tetracyclines and gentamycin.

(ii) Choice of medium

Consistent and reproducible results are obtained in media prepared especially for sensitivity testing the plates must be poured flat with an even depth, very thin plates are unsatisfactory.

(iii) Effect of pH

The activity of amino-glycosides is enhanced in alkaline media and reduced in acidic media, the reverse isshown by tetracyclin.

(iv) Size of inoculum

Although many antibiotics are not markedly affected by large number of organisms, all inhibition zones are diminished by heavy inocula. Overnight broth cultures of organisms and suitable suspensions from solid media can be diluted accurately to give optimum inocula for sensitivity testing. In pratice, satisfactory results can be achieved by taking a loopful of a well grown culture, or a suitably made suspension of organisms and spreading it with dry sterile Swab.[15]

The performance of diffusion technique

(i) Composition of nutrient agar

Peptone - 2 g; NaCl - 2 g.

Meat extract - 3.2 g; Agar-agar Powder - 8 g

pH - 7.4 ; Distilled water - 1000 ml.

(ii) Strength of antibiotics

Until very recently, there has been little or no agreement regarding the strength of antibiotic disks for use 'invitro' sensitivity tests.

(iii) Storage of disks

Disks should always be kept cool and dry and when applied to the medium should be pressed firmly to ensure proper contact and even diffusion disks may be applied to culture media very convenient with fine pointed forceps, dissecting needles or hypodermic needles.

(iv) Incubation time

It should ideally be the minimum required for the normal growth of the organism. Prolonged incubation of a culture may result in inactivation of the antibiotic and result in the subsequent growth of organism.

(v) Controls

For the correct interpretation of results and recognition of any source of error in disk diffusion

sensitivity tests, the correct use of control organism isessential. If multiple disks, necessitating the use of whole plates are used. Control plates should be set up for every drug and medium. For routine daily use, the organisms are most conveniently kept in a refrigerator at 4°C on sterile throat swabs, a jar full of such swabs can be impregnated at one time as they keep well at least a week.

The compounds described in Chapter-II to IV have been screened for antibacterial activity and tabulated in Tables-1 to 6 under the following heads:

(1) Mannich bases from Aminoacids

(2) Mannich bases from Amines via Chloromethylation

(3) Schiff's bases

(4) Oxadiazoles and Hydrazides

(5) Anilides and Amides

(6) Sulfonamides

N.B. : Tables 1 to 6

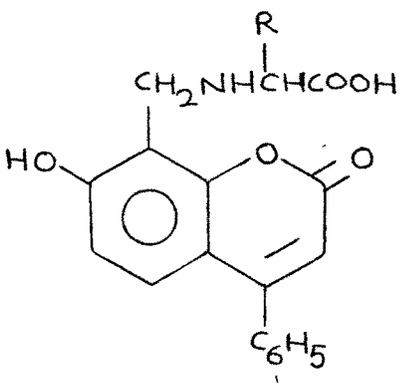
(i) (+) indicate zone diameter of growth inhibition in mm

\+ Zone diameter less than 15 mm

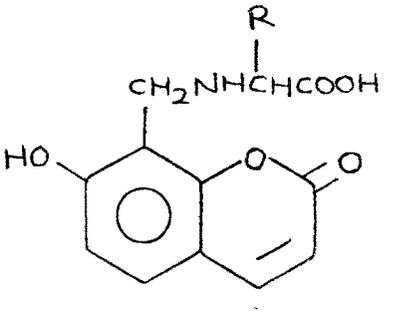
++ Zone diameter more than 15 mm.

(ii) (-) indicates no inhibitory zone around the disk.

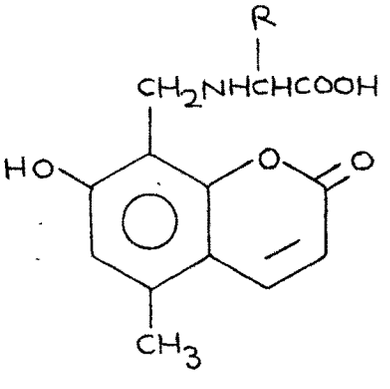
(iii) Solvent used : Dimethylformamide


R
CH2NHCHCOOH
HO
O
O
C6H5

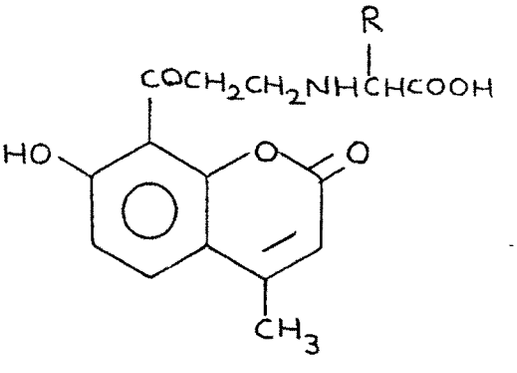
(I-A)


R
CH2NHCHCOOH
HO
O
O

(I-C)


R
CH2NHCHCOOH
HO
O
O
CH3

(I-B)

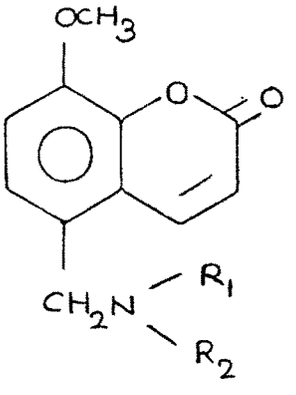

R
COCH2CH2NHCHCOOH
HO
O
O
CH3

(I-D)

TABLE-1 : ANTIBACTERIAL ACTIVITY OF MANNICH BASES FROM AMINOACIDS

St No	I	R	Interpretation zone of inhibition at 100 and 500 ppm conc.							
			E.Coli		S.aureus		S.typhosa		S.albus	
			100	500	100	500	100	500	100	500
A	1	$-CH_2CH_2SCH_3$	-	-	-	-	-	-	-	++
	2	$-CH(CH_3)_2$	-	-	-	-	++	++	-	-
	3	$-CH_2C_6H_5$	-	-	-	-	-	-	-	++
	4	-H	-	-	-	-	-	++	-	-
B	5	$-CH_2CH_2SCH_3$	-	-	-	-	-	-	-	-
	6	$-CH_3$	-	-	-	-	-	-	-	-
C	7	$-CH_2CH_2SCH_3$	-	-	-	-	-	-	-	-
	8	$-CH_2C_6H_5$	-	-	-	-	++	++	++	++
D	9	-H	-	-	-	-	-	-	-	-
	10	$-CH_2OH$	-	-	-	-	-	-	-	-

The activities were compared with phthalylsulfathiazole

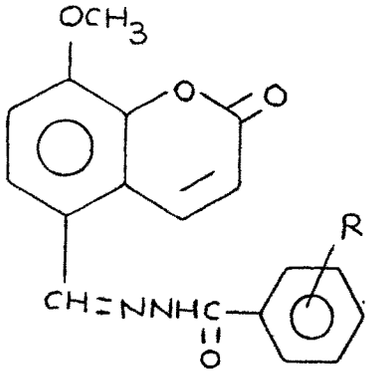


(II)

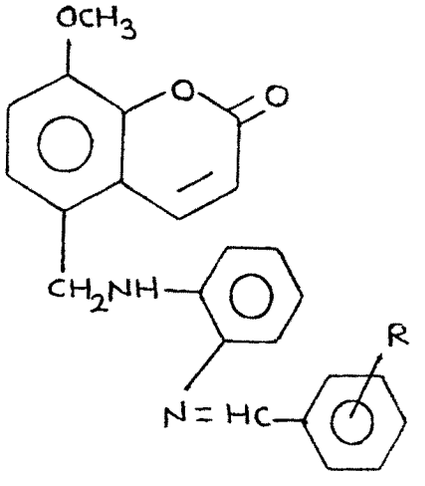
TABLE - 2 : ANTIBACTERIAL ACTIVITY OF MANNICH BASES FROM AMINES VIA CHLOROMETHYLATION

St No. II	R	R_1	Interpretation zone of inhibition at 100 and 500 ppm conc.							
			E.coli		S.aureus		S.typhosa		B.subtilis	
			100	500	100	500	100	500	100	500
1	H	C_6H_4-	-	-	-	-	-	-	-	-
2	H	o-$CH_3C_6H_4$-	-	-	-	-	-	-	-	-
3	H	o-OCH_3-C_6H_4-	-	-	-	-	-	-	-	-
4	H	p-Cl-C_6H_4-	-	-	-	+	-	-	-	-
5	H	p-Br-C_6H_4-	-	-	-	-	-	-	-	-
6	H	β-naphthyl	-	-	-	+	-	-	-	-
7	H	∝-naphthyl	-	-	-	-	-	-	+	++
8	H	p-$COOC_2H_5$-C_6H_4-	-	-	-	-	-	-	-	-
9	C_2H_5	C_6H_5	-	-	-	-	-	-	-	-
10	C_6H_5	C_6H_5	-	+	-	-	-	-	+	++

The activities were compared with ampicillin



(Ⅲ-A)



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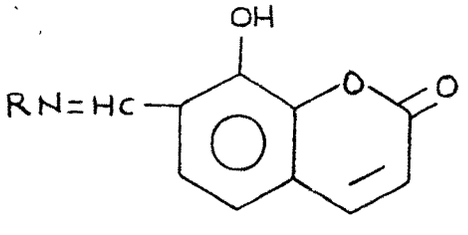

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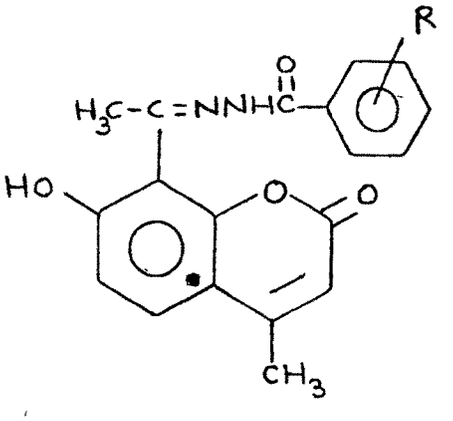
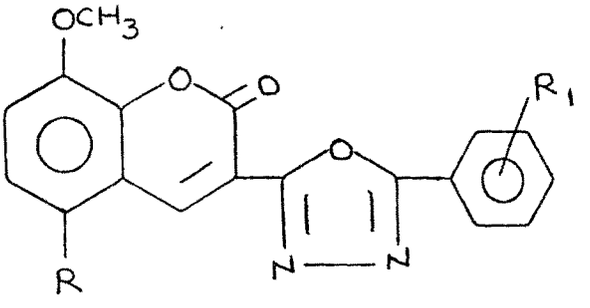


(Ⅲ-D)

TABLE - 3 : ANTIBACTERIAL ACTIVITY OF SCHIFF'S BASES

St No. III		R	Interpretation zone of inhibition at 100 and 500 ppm conc.							
			E.coli		S.aureus		S.albus		B.subtilis	
			100	500	100	500	100	500	100	500
A	1	m-Br	++	++	-	-	++	++	++	++
	2	p-Cl	-	++	+	+	-	-	-	-
	3	m-OCH_3-	-	++	+	++	++	++	++	++
	4	thiosemicarbazide	-	-	-	+	-	++	++	++
B	5	p-OH-	++	++	-	-	-	-	-	-
	6	p-Cl-	-	-	-	+	++	++	-	-
	7	o-NO_2-	-	++	-	-	-	-	-	-
	8	2-OH -5-Br-	-	-	-	+	++	++	-	-
C	9	(o-ClC_6H_4)-CONH-	++	++	-	-	++	++	-	-
	10	p-BrC_6H_4-	-	++	-	-	++	++	-	-
	11	p-$NO_2C_6H_4$-	-	+	-	-	++	++	-	+
D	12	p-NH_2-	+	++	-	-	-	-	-	-

The activities were compared with ampicilin



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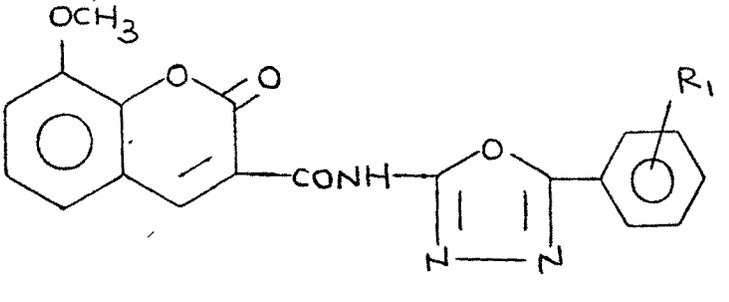
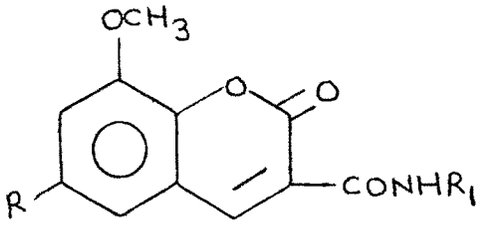


(Ⅳ-B)

TABLE - 4 : ANTIBACTERIAL ACTIVITY OF OXADIAZOLES

St No. IV		R	R_1	Interpretation zone of inhibition at 100 and 500 ppm conc.							
				E.coli		S.aureus		S.typhosa		S.albus	
				100	500	100	500	100	500	100	500
A	1	H	m-NO_2-	+	++	-	-	-	-	-	-
	2	H	p-NH_2-	-	-	-	-	-	-	-	-
	3	H	p-NO_2-	+	++	-	-	-	-	-	-
	4	Br	p-NO_2-	-	-	-	-	-	-	-	-
	5	Br	p-Cl-	-	-	-	-	-	-	-	-
	6	Br	m-Br-	++	++	++	++	-	++	-	-
	7	H	o-Cl-	-	-	-	-	-	++	-	-
	8	H	m-OCH_3-	+	++	++	++	-	-	-	-
B	9	H	p-Cl-	-	-	-	+	-	-	-	-
	10	H	m-OCH_3-	-	-	-	-	-	-	-	-

The activities were compared with ampicillin



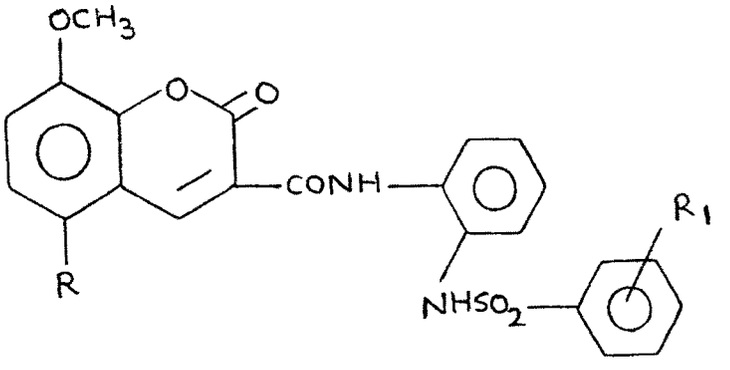
(V)

TABLE - 5 : ANTIBACTERIAL ACTIVITY OF ANILIDES AND AMIDES

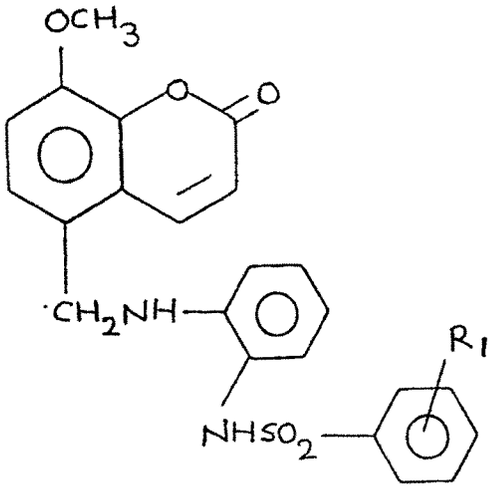
Solvent : DMF - Benzene

St. No. V	R	R^1	Minimum inhibitory conc. (MIC) in µg/ml against Bacteria					Fungi				
			1	2	3	4	5	6	7	8	9	10
1	H	o-Cl-C_6H_4-	-	-	-	-	-	-	-	-	-	-
2	H	p-Br-C_6H_4-	-	-	-	-	-	-	25	25	-	-
3	H	∝-naphthyl	-	-	-	-	-	-	-	-	-	-
4	H	p-Cl-C_6H_4-	-	-	-	-	-	-	-	-	-	-
5	H	p-$COOC_2H_5$-C_6H_4	-	-	-	-	-	-	-	-	-	-
6	H	m-CH_3-C_6H_4-	-	-	-	-	-	-	-	-	-	-
7	H	4-phenyl thiazole-2-	-	-	-	-	-	-	-	-	-	-
8	Br	p-Br-C_6H_4-	-	-	-	-	-	-	-	-	25	-

1. Streptococcus faecalis 2. Klebsiella pneumoniae 3. Escherichia coli. 4. Pseudomonas aeruginosa 5. Staphylococcus aureus Penicillin resistance (2500 units) 6. Candida albicans 7. CryptOcoccus neoformans 8. Sporotrichum schenkii 9. Trichophyton mentaqrophytes 10. Asperquillus fumigatus



(Ⅵ-A)



(Ⅵ-B)

TABLE - 6 : ANTIBACTERIAL ACTIVITY OF SULPHONAMIDES

St No. VI		R	R_1	Interpretation zone of inhibition at 100 and 500 ppm conc. E.Coli 100	E.Coli 500	S.aureus 100	S.aureus 500	S.typhosa 100	S.typhosa 500	S.albus 100	S.albus 500
A	1	H	p-Br-	-	-	-	-	-	-	-	+
	2	H	p-OCH_3-	-	-	-	-	-	-	-	-
	3	H	p-Cl-	-	-	++	++	+	++	++	++
	4	H	$NHCOCH_3$-	+	++	-	-	-	-	-	-
	5	H	p-CH_3-	+	++	-	-	-	-	-	-
	6	Br	p-Cl-	-	++	++	++	++	++	-	++
	7	Br	p-Br-	-	-	-	++	+	++	-	-
	8	Br	$NHCOCH_3$-	-	-	-	-	-	-	-	-
B	9	H	p-Cl-	-	+	+	+	-	+	++	++
	10	H	p-CH_3-	-	-	+	++	-	-	++	++
	11	H	$NHCOCH_3$-	-	+	-	-	-	-	-	-
	12	H	p-Br-	-	-	-	-	-	-	-	-

The activities were compared with ampicillin

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