SEARCH FOR PHYSIOLOGICALLY ACTIVE COMPOUNDS

Part XIII. Synthesis of Some 3-Amino-4-Hydroxy Coumarins and Coumarin (3 : 4) Oxazoles as Analogues of Novobiocin

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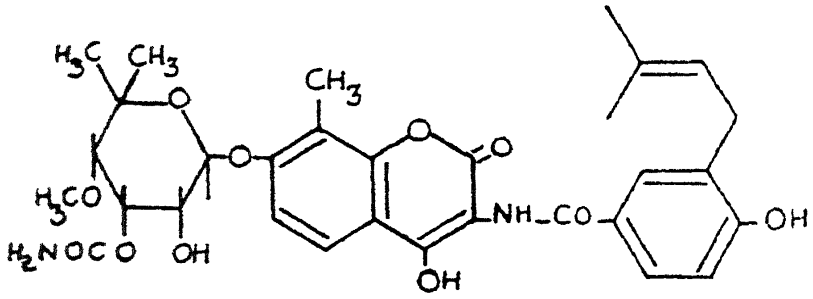
Abstract

A number of 3-amino-4-hydroxy coumarins substituted in the benzene ring have been synthesised by two methods starting from 4-hydroxy coumarins, one by nitration and reduction, and the other by diazotisation-coupling and reduction. The amino hydroxy coumarins have been converted into the corresponding (3 : 4) oxazoles by condensation with aromatic aldehydes. The ultraviolet and infrared spectral data as well as their bacteriostatic and fungistatic properties are presented.

The chemistry and physiological activity of 3-amino-4-hydroxy coumarin have acquired greater prominence in recent years with the isolation and study of the structure of the antibiotic Novobiocin (I).[1] The antibiotic has been reported to possess appreciable antitubercular activity[2] in addition to its fungicidal[3] and amoebicidal[4] activities. Several 3-amino 4-hydroxy coumarins and coumarino (3: 4) oxazoles were tested for antibacterial and antifungal properties.[5, 6] The preparation of a few N alkyl and aryl substituted 3-amino-4-hydroxy coumarins and coumarino (3: 4) oxazoles and their physiological activity have been described in an earlier communication from these laboratories.[7]

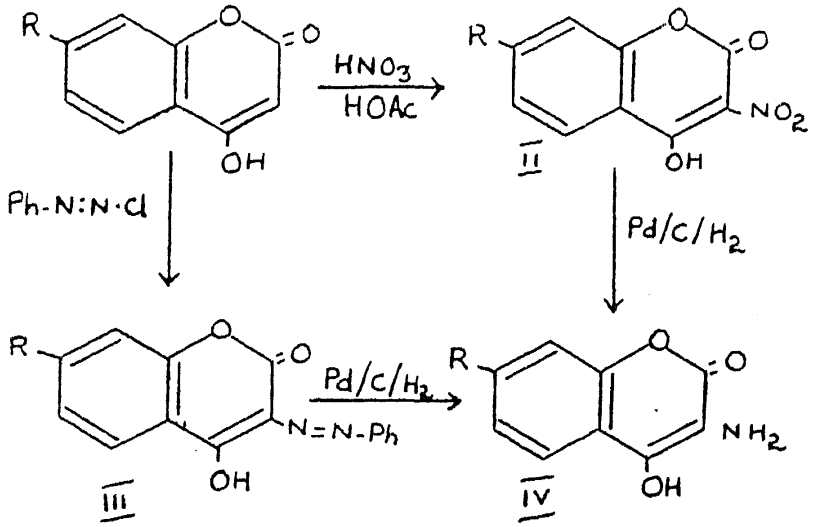


(I)

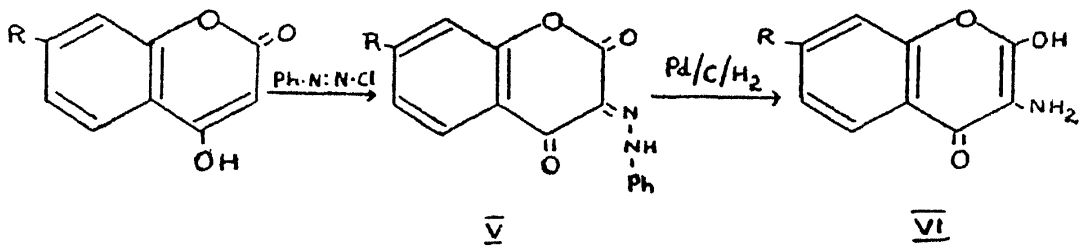
With a view to testing the bacteriostatic and fungistatic properties the synthesis of a few 3-amino-4-hydroxy coumarins and coumarino (3 : 4

oxazoles possessing substituents in the benzenoid portion of the coumarin moiety has been undertaken. The starting materials necessary are 4-hydroxy coumarins, which have been obtained by the Boyd-Robertson[8] method, making use of appropriately substituted *o*-hydroxy acetophenones. 4: 7-Dihydroxy coumarin, on the other hand, has been synthesised by the Hoesch reaction starting from resorcinol and ethyl cyanoacetate.[9]

For the preparation of 3-amino-4-hydroxy coumarins (IV), two methods[10] have been adopted, starting from a 4-hydroxy coumarin (i) by nitration to the corresponding 3-nitro derivative (II) and subsequent reduction by hydrogenation over palladium on carbon catalyst and (ii) by treatment with diazotised aniline resulting in the 3-phenyl azo derivative (III) which was later reduced by the same procedure adopted for the reduction of the nitro compound.

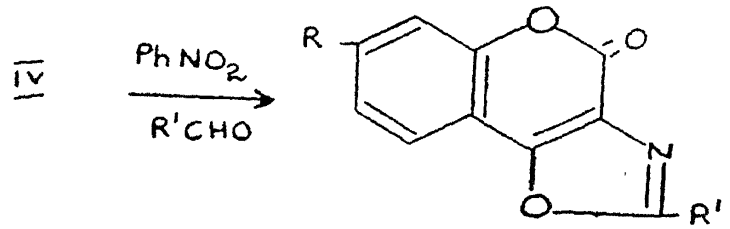


Okumura[11] assigned a 2: 3: 4-trioxo chroman-3-phenylhydrazone type of structure (V) to the products of condensation of 4-hydroxy coumarins with phenyl diazonium chloride and a 2-hydroxy chromone type of structure (VI) to the products of reduction of these compounds.



However, it was subsequently shown by workers from the Hoffmann-La Roche Company[12] that the products formed by the condensation of

4-hydroxy coumarins with diazotised aniline were 3-phenyalzo-4-hydroxy coumarins and the reduction products were 3-amino-4-hydroxy coumarins. This observation was supported by the present work in which the products of reduction of both the 3-nitro and the 3-phenylazo derivatives have been found to be 3-amino-4-hydroxy coumarins only. 7-Hydroxy, 7-chloro, 7-bromo, 7-acetamino, 7-methoxy, 5-methoxy and 6-methyl 3-amino-4-hydroxy coumarins have been prepared by both the methods (Table I). These 3-amino-4-hydroxy coumarins were converted into the corresponding coumarino (3:4) oxazoles (VII) by refluxing with an aromatic aldehyde in nitrobenzene medium. The coumarino-oxazoles synthesised are included in Table II.



VII

All these compounds have been tested for bacteriostatic and fungistatic properties. The bacteriostatic activity has been elucidated employing the tube dilution method using *B. coli*, *Sta. aureus* and *B. subtilis* as the testing organisms. The fungistatic activity has been determined by the reduction in radial growth employing *Aspergillus niger* as the test organism. The results are presented in Table III.

Among the amino hydroxy coumarins, the 7-chloro and the 7-bromo analogues have shown appreciable activity, in keeping with the observation earlier recorded in our laboratories that 7-chloro and 7-bromo coumarins possess appreciable activity. Among the oxazoles also, the 7-halo compounds have exhibited appreciable activity. A notable feature is the uniform activity exhibited by oxazoles with a "2'-(9-anthryl) substituent".

The ultraviolet and infrared spectra of a few coumarino (3:4) oxazoles have been recorded. In the ultraviolet region, the oxazoles exhibited a uniform band around $255 \pm 5\ m\mu$. In the infrared region, a band around $5{\cdot}9$–$6{\cdot}0\ \mu$ has been observed in all the cases, characteristic of the coumarin carbonyl, in addition to bands at $6{\cdot}5\ \mu$, $7{\cdot}1\ \mu$, $8{\cdot}0\ \mu$ and $9{\cdot}5\ \mu$, characteristic of the oxazoles.

TABLE I

S. No.	Starting material (Coumarin)	3-Nitro derivative			3-Phenylazo derivative			3-Amino-4-hydroxy coumarin		
		M.P. (° C.)	% Nitrogen		M.P. (° C.)	% Nitrogen		M.P. (° C.)	% Nitrogen	
			Calculated	Found		Calculated	Found		Calculated	Found
1	4 : 7-Dihydroxy[9] ..	174 (*d*)	6·3	6·2	273[13,14]	..	..	227	6·1	6·1
2	4-Hydroxy-7-chloro[15] ..	178 (*d*)	5·8	6·0	218	9·2	9·3	241	5·6	5·5
3	4-Hydroxy-7-bromo[15] ..	184 (*d*)	4·9	4·8	205	8·4	8·1	248	4·7	4·8
4	4-Hydroxy-7-methoxy[16] ..	167 (*d*)	5·9	5·6	215	9·5	9·4	218	5·8	5·7
5	4-Hydroxy-7-acetamino[17] ..	170 (*d*)	10·6	10·5	190	12·7	12·5	195	10·2	10·4
6	4-Hydroxy-6-methyl[18] ..	171 (*d*)	6·3	6·2	191[19]	..	..	167	6·2	6·2
7	4-Hydroxy-5-methoxy[20] ..	167 (*d*)	5·9	6·0	202	9·5	9·6	135	5·8	5·6

TABLE II

Sl. No.	Coumarino (3 : 4) oxazole	M.P. (° C.)	Yield (%)	% Nitrogen	
				Cal.	Found
1.	7-Hydroxy-2-phenyl	169	55	5·0	4·9
2.	7-Hydroxy-2-(9-anthryl)	155	55	3·7	3·6
3.	7-Hydroxy-2-(*p*-anisyl)	141	60	4·5	4·4
4.	7-Hydroxy-2-vanillyl	261	50	4·3	4·2
5.	7-Hydroxy-2-*p*-dimethylaminophenyl	98	50	8·7	8·8
6.	7-Methoxy-2-(9-anthryl)	140	50	3·6	3·5
7.	7-Methoxy-2-(2-ethoxy-1-naphthyl)	114	50	3·6	3·5
8.	7-Chloro-2-(9-anthryl)	108	50	3·5	3·4
9.	7-Chloro-2-(2-ethoxy-1-naphthyl)	105	50	3·6	3·6
10.	7-Chloro-2-*p*-anisyl	171	90	4·3	4·1
11.	7-Bromo-2-(9-anthryl)	109	45	3·2	3·1
12.	7-Bromo-2-(2-ethoxy-1-naphthyl)	108	60	3·2	3·0
13.	7-Acetamino-2-(*p*-anisyl)	145	70	8·0	7·9
14.	7-Acetamino-2-(*p*-dimethylaminophenyl)	101	45	11·0	10·9
15.	7-Acetamino-2-(9-anthryl)	111	50	6·7	6·6
16.	6-Methyl-2-(*p*-dimethylaminophenyl)	159	50	8·8	8·7
17.	6-Methyl-2-(9-anthryl)	169	60	3·7	3·6
18.	5-Methoxy-2-(2-ethoxy-1-naphthyl)	118	60	3·7	3·6
19.	5-Methoxy-2-(*p*-anisyl)	137	35	4·3	4·2

EXPERIMENTAL

(*a*) *General procedure for the nitration of 4-hydroxy coumarins.*—The 4-hydroxy coumarin (2 g.) was suspended in glacial acetic acid (10 ml.) to which was then added a mixture of concentrated nitric acid (1 ml.) and

TABLE III

Physiological activity

S. No.	Coumarin	Bacteriostatic activity						Fungistatic activity	
		B.C.		S.A.		B.S.		% Inhibition of *Aspergillus niger*	
		a	*b*	*a*	*b*	*a*	*b*	*a*	*b*
	A. 3-Amino-4-hydroxy coumarins:								
1	3-Amino-4: 7-dihydroxy	+	−	+	−	+	−	25	70
2	3-Amino-4-hydroxy-7-chloro	−	−	−	−	−	−	55	100
3	3-Amino-4-hydroxy 7-bromo	−	−	−	−	−	−	55	100
4	3-Amino-4-hydroxy-7-methoxy	+	−	±	−	−	−	25	80
5	3-Amino-4-hydroxy-7-acetamino	+	±	+	±	+	±	10	50
6	3-Amino-4-hydroxy-6-methyl	+	−	+	−	+	−	20	65
7	3-Amino-4-hydroxy-5-methoxy	+	−	+	−	+	−	15	60
	B. Coumarino (3 : 4) oxazoles:								
1	7-Chloro-2-(*p*-anisyl)	−	−	−	−	−	−	25	65
2	7-Chloro-2-(9-anthryl)	−	−	−	−	−	−	55	90
3	7-Chloro-2-(2-ethoxy-1-naphthyl)	−	−	−	−	−	−	45	80
4	7-Bromo-2-(9-anthryl)	−	−	−	−	−	−	55	90
5	7-Bromo-2-(2-ethoxy-1-naphthyl)	−	−	+	−	−	−	45	80
6	7-Methoxy-2-(9-anthryl)	±	−	±	−	±	−	10	55
7	7-Hydroxy-2-(9-anthryl)	±	−	±	−	±	−	0	40
8	7-Acetamino-2-(9 anthryl)	+	−	+	−	+	−	25	60
9	7-Hydroxy-2-vanillyl	+	−	+	−	+	−	0	35
10	7-Hydroxy-2-*p*-anisyl	+	+	+	+	+	+	25	65

+ Full growth; − No growth; ± Partial growth.

a = 1 in 100,000 dilution; *b* = 1 in 10,000 dilution.

Note.—The other coumarino exazoles are inactive even at 1 in 10,000 dilution.

glacial acetic acid (5 ml.). The mixture was warmed on a water-bath at 80° C. at which temperature, nitration suddenly began. The mixture was immediately cooled in ice, the solid filtered and washed with the minimum amount of alcohol and recrystallised from alcohol.

(*b*) *General procedure for diazotised aniline coupling.*—To a suspension of the 4-hydroxy coumarin (3 g.) in ethanol (20 ml.) was added crystalline sodium acetate (15 g.) and water (10 ml.). The mixture was cooled to 0° C. and with stirring, a diazonium solution prepared from aniline (2 g.) in hydrochloric acid (10 ml., in 20 ml. water) was added. The stirring was continued for one hour and the precipitated compound filtered and washed thoroughly with water until free from acid.

(*c*) *General procedure for the reduction of nitro and azo compounds.*—The nitro compound or the 3-phenyl azo derivative was suspended in methanol (100 ml.) to which was then added freshly prepared palladium charcoal catalyst. In the case of the nitro compound, a few ml. of 1% methanolic hydrogen chloride was also added. The mixture was hydrogenated at atmospheric pressure for 3 hours and filtered. The filtrate was evaporated in the case of the nitro compound. The reduction product of the 3-phenyl azo compound was separated from the catalyst by treatment with ethanol-hydrochloric acid mixture (4:1) and the 3-amino compound was obtained on concentrating the solution. The 3-amino-4-hydroxy coumarin was recrystallised from methanol.

(*d*) *Preparation of coumarino* (3:4) *oxazoles.*—The 3-amino-4-hydroxy coumarin (1 g.) was refluxed with an aromatic aldehyde (1 g.) in nitrobenzene (25 ml.) for 3 hours. The excess of nitrobenzene and unreacted aldehyde were removed by steam distillation and the residue was recrystallised from benzene-petroleum ether mixture and finally subjected to chromatography over alumina.

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