SEMISYNTHETIC COUMERMYCINS. VI

PREPARATION AND PROPERTIES OF 3-(3-ARYL- AND 3-ARALKYL-4-HYDROXYBENZAMIDO)-4-HYDROXY-8-METHYL-7-[3-O-(5-METHYL-2-PYRROLYLCARBONYL)NOVIOSYLOXY]COUMARINS

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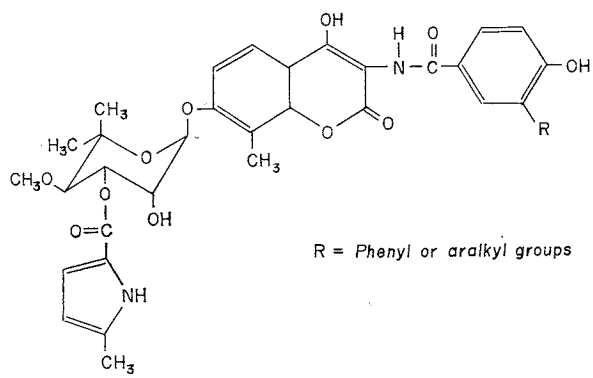
A series of new antibiotics was derived from coumermycin A_1 by reaction with 4-acetoxy-3-aryl and 3-aralkyl benzoyl chlorides. The compounds with a 3-benzyl-4-hydroxybenzamido or a 3-β-phenylethyl-4-hydroxybenzamido side chain inhibited Gram-positive bacteria at very low concentrations *in vitro*, protected mice against infection, and gave satisfactory blood levels in dogs on oral dosing.

In an attempt to improve the pharmacological properties of semisynthetic coumermycins, we extended the work previously reported[1~6] to the preparation of compounds shown in Fig. 1.

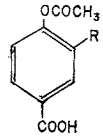
Fig. 1



These compounds, particularly the 3-benzyl (Fig. 1, R=$-CH_2C_6H_5$) and the 3-β-phenylethyl (Fig. 1, R=$-CH_2CH_2C_6H_5$) derivatives, inhibited many strains of bacteria at lower concentrations than the compounds described in the earlier communications and gave useful blood levels in dogs after oral dosing.

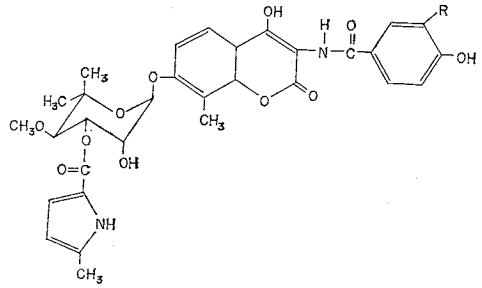
The requisite 4-acetoxybenzoic acids are listed in Table 1. The corresponding acid chlorides were reacted with di-tetrahydropyranylcoumermycin A_1 as described by KEIL *et al.*[1,2] The antibiotically active compounds were obtained by cleavage of the tetrahydropyranyl ethers, followed by deacetylation in liquid ammonia[4].

Table 1. Precursor acids for coumermycin derivatives



R	m. p. (°C)	Formula	Calculated		Found	
			% C	% H	% C	% H
C_6H_5	184~186	$C_{15}H_{12}O_4$	70.30	4.72	70.26	4.77
$C_6H_5CH_2$	165~166	$C_{16}H_{14}O_4$	71.10	5.22	71.12	5.44
$C_6H_5CH_2CH_2$	178	$C_{17}H_{16}O_4$	71.82	5.67	71.98	5.76
2-$ClC_6H_4CH_2$	175~179	$C_{16}H_{13}O_4C$	63.06	4.30	63.39	4.47
4-$ClC_6H_4CH_2$	154	$C_{16}H_{13}O_4Cl$	63.06	4.30	62.93	4.43
$C_6H_5CHCH{=}CH_2$	122~128	$C_{18}H_{16}O_4$	72.96	5.44	72.22	5.25

Table 2. Semisynthetic coumermycin derivatives



Compound No.	R	Dec. Pt. (°C)	Calculated		Found	
			% C	% H	% C	% H
1	C_6H_5	213	64.90	5.30	64.57	5.67
2	$C_6H_5CH_2$	182	65.32	5.48	65.00	5.71
3	$C_6H_5CH_2CH_2$**	215	64.90	5.73	65.14	5.71
4	2-$ClC_6H_4CH_2$	140	62.25	5.09	62.53	5.58
5	4-$ClC_6H_4CH_2$	198	62.25	5.09	62.29	5.82
6	$C_6H_5CHCH{=}CH_2$**	166	65.47	5.63	65.62	5.84
7*	$C_6H_5CHCH_2CH_3$	145	66.10	5.82	65.87	6.08

* Prepared by catalytic reduction of Compound 6. ** $^1/_2\,H_2O$

The physical data for the new antibiotics are presented in Table 2, and Tables 3 and 4 list some of the biological properties. All intermediates gave satisfactory infrared and nmr spectra, and the final products appeared homogeneous on thin-layer chromatography.

Experimental*

4-Acetoxy-3-phenylbenzoic acid

A mixture of acetic acid (1 mole) and 2-hydroxybiphenyl (1.25 mole) was saturated

* For literature citations of the general procedures used see reference (4).

Table 3. Minimum inhibitory concentrations and oral CD_{50}'s of semisynthetic coumermycin derivatives

Compound No.	*Diplococcus pneumoniae* +5% serum	*Streptococcus pyogenes*	*Staphylococcus aureus*				*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	CD_{50} *vs. S. aureus* Smith mg/kg P. O. (mice)
			Smith	Smith +50% serum	Novobiocin resistant	Coumermycin resistant			
1	12.5	1.6	0.062	50	6.2	0.8	100	100	12.5
2	0.06	0.008	0.003	1.6	0.4	0.06	12.5	3.1	8.0
3	1.6	0.062	0.003	0.8	0.4	0.06	3.1	25	1.3
4	3.1	0.2	0.002	12.5	0.8	0.2	100	50	13.0
5	0.4	0.2	0.016	12.5	0.8	0.2	100	>100	15.0
6	3.1	0.2	0.002	25	0.4	6.2	25	100	11.5
7	3.1	0.2	0.002	>50	0.4	0.2	12.5	50	9.0
Novobiocin	3.1	0.8	0.062	6.2	25	0.8	25	>100	10
Coumermycin	0.31	0.08	0.0008	1.6	6.2	0.8	3.1	25	12

Table 4. Oral blood levels in Beagle dogs, 25 mg/kg*

	Blood levels (μg/ml)				
	1 hr.	2 hrs.	4 hrs.	7 hrs.	24 hrs.
Compound 2	13	14	11	6	2
Compound 3	14	35	32	24	5
Coumermycin A_1	2	5	5	4	<1
Novobiocin	74	83	38	6.9	—

* Average of five dogs.

with boron trifluoride for two hours at 65~68°C to yield 4-hydroxy-3-phenylacetophenone in 60% yield, m. p. 173~174°C.

Analysis. Calculated for $C_{14}H_{12}O_2$: C 79.22, H 5.70.
Found : C 79.24, H 5.80.

To the acetophenone (10.6 g) in pyridine (20 ml) was added iodine (12.7 g), the mixture heated on a steam bath for 45 minutes, and left at room temperature for 20 hours. The product was acidified, transferred into ether, and extracted into aqueous sodium bicarbonate. On acidification 4-hydroxy-3-phenylbenzoic acid precipitated (8.1 g). Treatment with pyridine-acetic anhydride gave the acetoxy acid which after three recrystallizations from benzene melted at 184~186°C (Compound 1, Table 1).

4-Acetoxy-3-benzylbenzoic acid

Ethyl 4-hydroxybenzoate (32.2 g, 0.2 mole) in toluene (75 ml) was added dropwise to a stirred suspension of sodium hydride (4.8 g, 0.2 mole) in toluene (30 ml). To this mixture, maintained at gentle reflux, was added benzyl chloride (28 g, 0.22 mole). The reaction was completed by vigorous boiling under reflux for five hours. After cooling, about 50 ml of 1 N hydrochloric acid was added, the toluene layer separated and washed with water (30 ml) and 5% aqueous sodium bicarbonate (3×40 ml). The hydroxy ester was then extracted with 5% sodium hydroxide solution (3×50 ml). The aqueous layers were acidified with 6 N hydrochloric acid and extracted with ether. On drying, 10.4 g of the crude ester was obtained. The ester (9.4 g) was boiled for two hours under reflux with 200 ml of 1 N methanolic potassium hydroxide. The solution was acidified and distilled to a small volume and yielded 6.5 g of the free acid, m. p. 131~132°C. The acid (5 g) was treated with pyridine (40 ml) and acetic anhydride (20 ml) for 16 hours at room temperature. The solution was poured onto ice (200 g) and extracted with ether (3×125 ml). The ethereal solution was extracted into 5% aqueous sodium bicarbonate (3×100 ml). Acidifi-

cation afforded the acetoxy acid in quantitative yield (Compound 2, Table 1).

4-Acetoxy-3-β-phenylethylbenzoic acid

Ethyl 4-hydroxybenzoate (0.2 mole) and phenylacetyl chloride (0.2 mole) in acetylene tetrachloride (300 ml) was heated with aluminum chloride (56 g) to 120°C for $3^1/_2$ hours. The mixture was steam-distilled, and the residue heated with sodium carbonate (10 g) for two hours. Purification by transfer between ether and water and recrystallization from ethanol afforded in 10 % yield 4-hydroxy-3-phenylacetylbenzoic acid: m. p. 200°C.

Analysis. Calculated for $C_{15}H_{12}O_4$: C 70.30, H 4.72.
Found: C 69.92, H 4.98.

Clemmensen reduction yielded 4-hydroxy-3-β-phenylethylbenzoic acid; recrystallized from ethanol, m. p. 132°C, in 50 % yield.

Analysis. Calculated for $C_{15}H_{14}O_3$: C 74.36, H 5.83.
Found; C 74.64, H 5.73.

Alternatively, 2'-acetoxy-1, 2-diphenylethane (14.6 g) was treated in nitrobenzene (70 ml) with aluminum chloride (9.5 g) for 18 hours. The mixture was poured over ice (500 g) and steam distilled. The residue was extracted with ether (3×100 ml), and the ether extracted with 1 N sodium hydroxide (2×75 ml). On acidification 3-β-phenylethyl-4-hydroxyacetophenone (8.7 g) precipitated (m. p. 118~119°C).

Analysis. Calculated for $C_{16}H_{16}O_2$: C 79.97, H 6.71.
Found: C 80.05, H 6.77.

The ketone (7 g) was dissolved in pyridine (40 ml) and heated with iodine (7.6 g) to 100°C for one hour. After standing for 16 hours the mixture was washed with ether and heated to 100°C with aqueous sodium hydroxide (7 g in 350 ml) for one hour. The solution was acidified, extracted with ether, and the ether was extracted with sodium bicarbonate solution. Acidification yielded the acid (4.7 g).

4-Acetoxy-3-(2-chlorobenzyl)benzoic acid

Ethyl *p*-hydroxybenzoate and *o*-chlorobenzoyl chloride gave *via* the FRIEDEL-CRAFTS reaction as above 3-(2-chlorobenzoyl)-4-hydroxybenzoic acid, m.p. 134~135°C in 50 % yield. CLEMMENSEN reduction afforded 3-(2-chlorobenzyl)-4-hydroxybenzoic acid, m. p. 116~118°C which was acetylated to give Compound 4, Table 1, in 63 % yield.

4-Acetoxy-3-(4-chlorobenzyl)benzoic acid

Propyl *p*-hydroxybenzoate (60 g) was suspended in toluene (600 ml), sodium hydride (13.3 g of 59 % NaH in mineral oil) was added, the suspension heated to boiling, and *p*-chlorobenzyl chloride (53.2 g) added over a period of one hour. After boiling at reflux for 6 hours the product was extracted into aqueous sodium hydroxide (3×160 ml of 10 % NaOH). Hydrolysis gave 4-hydroxy-3-(4-chlorobenzyl)benzoic acid, m. p. 165°C, in 53~60 % yield.

Analysis. Calculated for $C_{14}H_{11}O_3Cl$: C 64.01, H 4.22.
Found: C 64.32, H 4.48, Cl 13.23.

Acetylation yielded Compound 5, Table 1, in almost quantitative yield.

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