SEMISYNTHETIC COUMERMYCINS. I

PREPARATION OF 3-ACYLAMIDO-4-HYDROXY-8-METHYL-7-[3-O-(5-METHYL-2-PYRROLYLCARBONYL)NOVIOSYLOXY]COUMARINS*

J. G. KEIL, I. R. HOOPER, M. J. CRON, O. B. FARDIG, D. E. NETTLETON, F. A. O'HERRON, E. A. RAGAN, M. A. ROUSCHE, H. SCHMITZ, R. H. SCHREIBER and J. C. GODFREY

Department of Biochemical Research, Bristol Laboratories, Division of Bristol-Myers Company, Syracuse, New York, U. S. A.

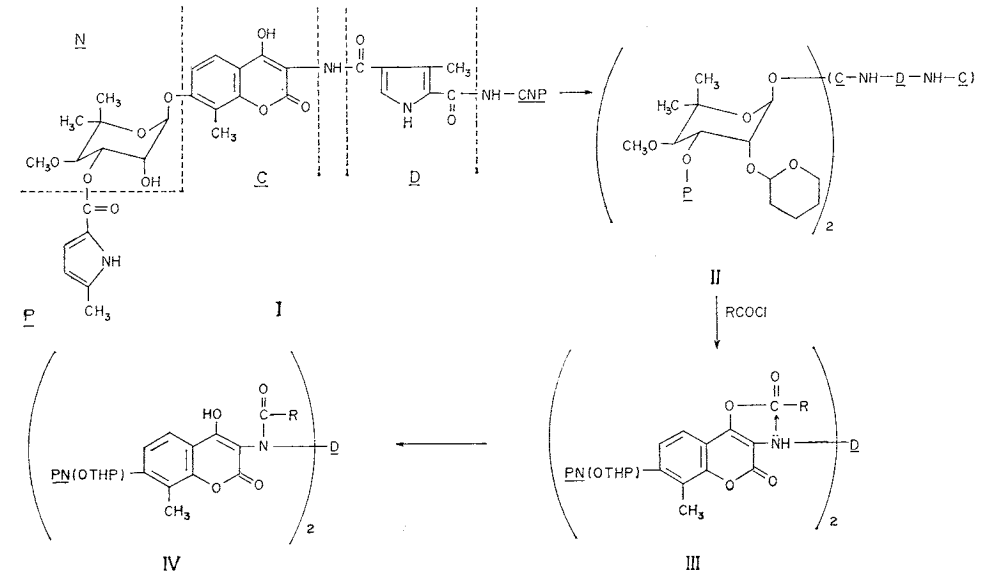
(Received for publication August 29, 1968)

The ditetrahydropyranyl ether of coumermycin A_1 was prepared and converted to a series of new derivatives by an acyl interchange reaction. The mechanism of this interesting and useful reaction is discussed. All of the 67 semisynthetic coumermycins described are active antibiotics, and several of them show desirable changes in physical properties from those of the parent coumermycin A_1. Comparative activities against *Staphylococcus aureus* SMITH are reported.

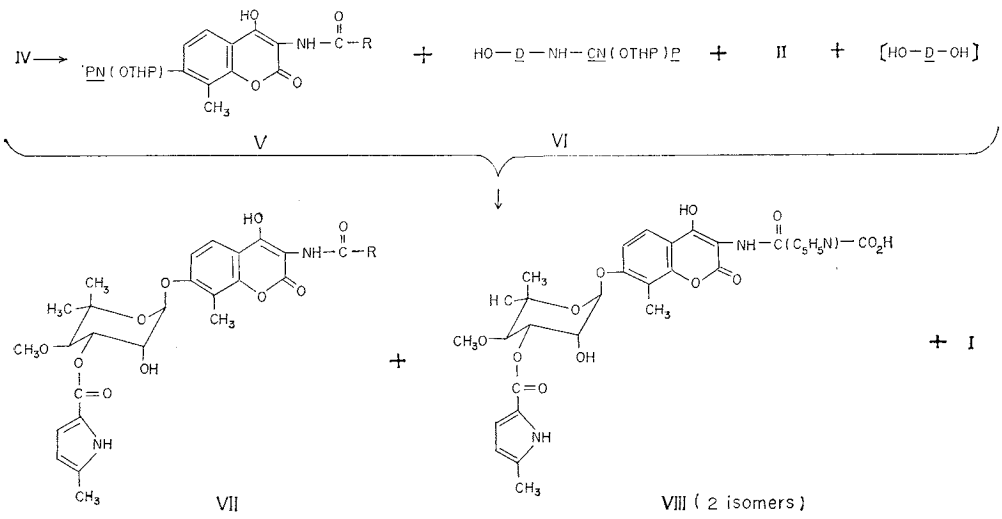
The complete structure of coumermycin A_1 (I) was first disclosed in a U.S. patent to KAWAGUCHI *et al.*[4] It was early recognized that coumermycin A_1 is a powerful and non-toxic antibiotic with a very good spectrum of activity against gram-positive organisms, as well as considerable activity against a variety of gram-negative bacteria[5]. In spite of a high degree of *in vitro* activity, it soon became apparent that because of the insolubility of coumermycin A_1, even as the sodium salt, parenteral administration was unsatisfactory. Poor oral absorption was also observed. The relationship of coumermycin A_1 to novobiocin, an antibiotic which is well absorbed orally, was apparent. Novobiocin contains the same association of sugar (noviose, N) and coumarin (2-R-amino-4-hydroxy-7-glycosidyl-8-methylcoumarin, C) moieties as is present in coumermycin A_1 (see Sequence A), but has a simple carbamoyl function, $-CONH_2$, in place of the 5-methylpyrrolyl-2-carbonyl group (P), and a 4-hydroxy-3-(3-methyl-2-butenyl)benzamide in place of the 3-methylpyrrole-2,4-dicarboxamide CNP (D-NH-CNP) of coumermycin A_1. It seemed that replacement of the 3-methylpyrrole-2,4-dicarboxamide with a simpler amide might result in a structure which would have many of the desirable features of coumermycin A_1, but which might in addition be more soluble and more readily absorbed *via* the oral route. The problem therefore reduced to that of removal of the D moiety and replacement of it with other acyl functions.

A clue to the displacement of D by other acyl functions was provided by the

* In the interest of brevity, "acylamido" represents substituted benzoic and the vinylogous cinnamic acid amides.

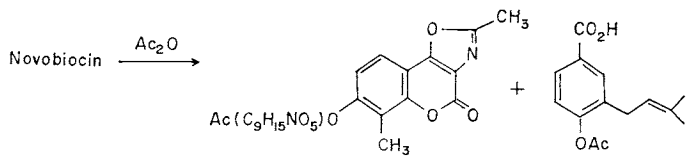


Sequence A, Part I



Sequence A, Part II

work of HINMAN *et al.*[3], on the degradation of novobiocin. They noted that novobiocin is cleaved by hot acetic anhydride to give 4-acetoxy-3-(3-methyl-2-butenyl) benzoic acid and an oxazole:

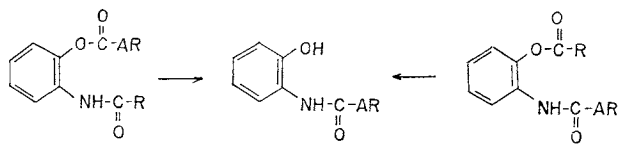


We concluded that it should, in principle, be possible either to find conditions for the acetylation which would result in displacement of the D moiety, or to hydrolyze

the oxazole to the 3-acetamido-4-hydroxycoumarin derivative without disruption of the rest of the molecule. In practice, the first-mentioned route was found to be the more practical, but resulted in a derivative in which the 2-hydroxyl of the noviose was blocked with an acetyl group. Derivatives having any sort of block on the noviose-2-hydroxyl have no antibiotic activity. Thus it was necessary to find a blocking group for this hydroxyl which could be readily put on and taken off in high-yield steps. The tetrahydropyranyl group met these requirements quite satisfactorily.

A suspension of coumermycin A_1 in a mixture of tetrahydrofuran and dihydropyran was treated with a catalytic amount of *p*-toluenesulfonic acid monohydrate and stirred at room temperature for several hours. Work up of the reaction mixture gave an 84% yield of 2',2'-O,O-ditetrahydropyranylcoumermycin A_1 (II), as fine, cubic crystals which decomposed above 200°C. Coumermycin A_1 was shown to be recoverable from the di-THP derivative in high yield by simply stirring it for several hours in methanol solution in the presence of a catalytic amount of *p*-toluenesulfonic acid monohydrate.

The next proplem was that of finding conditions for the replacement of the D moiety with less complex amides. A review of the literature on O- and N-acylated 2-aminophenols was very instructive. BELL[1] has reported that alkaline hydrolysis of

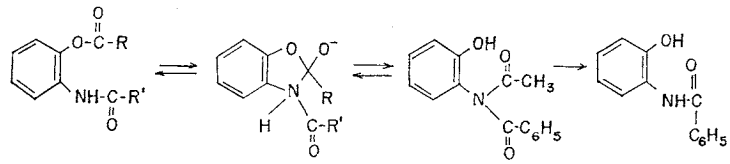


IXa, R=alkyl
AR=C_6H_5
IXb, R=C_6H_5
AR=α-naphthyl

Xa, AR=C_6H_5
Xb, AR=α-naphthyl

XIa, R=alkyl
AR=C_6H_5
XIb, R=C_6H_5
AR=α-naphthyl

compound IXa and IXb or XIa and XIb leads only to Xa and Xb. Since the product, X, is the same regardless of which group is initially on O and which on N, BELL postulated the following mechanism (using benzoyl acetyl compounds as examples):



R=C_6H_5 when R'=CH_3
and
R=CH_3 when R'=C_6H_5

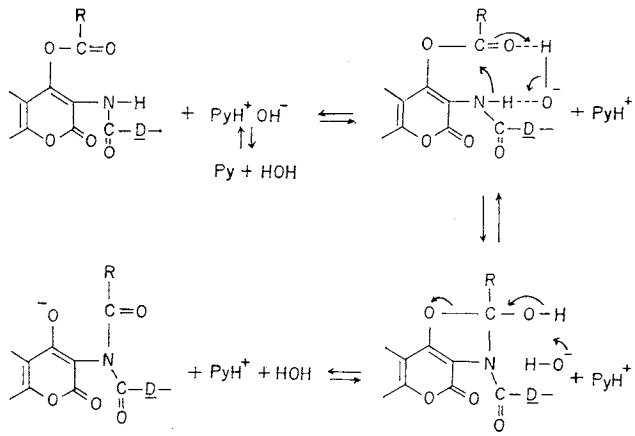
XII

Thus, whichever acyl group of the intermediate XII is the more susceptible to nucleophilic displacement will be the one which is displaced to give the observed *ortho*-acylaminophenol*. Several conditions were then found which permitted the

* For convenience, this reaction applied to coumermycin will hereinafter be referred to as the transacylation reaction.

conversion of *bis*-tetrahydropyranyl coumermycin A_1 (II) to the imide intermediate IV*. It is believed that this reaction occurs by way of the phenolic ester III, but in all cases III appeared to be very unstable with respect to IV, and IV was the first intermediate beyond II which could be isolated and fully characterized (see Experimetal).

The usual procedure consisted of the addition of four to ten molar equivalents of acid chloride or anhydride to a solution of tetrahydropyranyl coumermycin A_1 (II) in pyridine and stirring the mixture at a temperature between 25° and 115°C (reflux) for several hours. An unexpected finding was the beneficial effect of water upon some of the transacylation reactions. In the course of scaling up some of the preparations for pilot plant operation, it was found that very poor yields of transacylation product were obtained when absolutely anhydrous conditions were maintained. Eventually it was found that addition of two moles of water per mole of tetrahydropyranyl coumermycin A_1 gave the optimum yield of the desired product. (This observation was due to Drs. D. A. JOHNSON and E. J. RICHARDSON of the Chemical Development Department, Bristol Laboratories.) This observation may be explained as a specific catalysis of the transacylation reaction by hydroxide ion:



It is clear that pyridine itself could not provide this same kind of specific catalysis. A similar specific catalysis by bicarbonate and dihydrogen phosphate ions in the hydrolysis of 4-hydroxy-butyranalide under very mild conditions has recently been demonstrated[2].

In addition to excellent spectroscopic evidence for the proposed structures of the

* Considerable investigation was directed toward finding the best conditions for *bis* imide (IV) formation and ultimate displacement of the D moiety using the acylating reagents acetic anhydride, benzoic anhydride, and benzoyl chloride in pyridine as a solvent. It was found that the lower temperatures (25° to 50°C) and/or smaller molar ratios of reagent to ditetrahydropyranyl coumermycin A_1 (5 or less) favored the desired reaction. Higher temperatures (at or near reflux) and/or high ratios of reagent to ditetrahydropyranyl coumermycin A_1 (ca. 10, or greater) favored formation of 2-methyloxazoles and 2-phenyloxazoles analogous to that produced from novobiocin. As experience was gained with other reagents, it was noted that the most favorable results were obtained under mild conditions when a highly reactive acid chloride, such as *p*-nitrobenzoyl chloride, was used, whereas more drastic conditions gave the best results with reagents of low reactivity.

bis imides IV (*i.e.*, the absence of amide-II (NH), vibrations near 1540 cm^{-1} in their infrared spectra, the presence of the methyl group on the D moiety as shown by its typical nmr band at 2.4 ppm, the expected ratio between aromatic and methyl protons in the integrated nmr spectra, as well as satisfactory C, H, and N analyses), further support for these structures was found in the preparation under very mild conditions, of the PNC-dibenzoylimide. This compound was comparable in all expected respects to the intact *bis* imides IV. It had no amide-II (NH) band, and the ratio of aromatic to aliphatic protons in its integrated nmr spectrum was exactly as anticipated for PNC-dibenzoylimide.

As an alternative to the pyridine procedure, tetrahydropyranyl coumermycin A_1 was dissolved in tetrahydrofuran and treated at 0° to 25°C sequentially with five molar equivalents of triethylamine and four molar equivalents of an acid chloride. In several cases the *bis* imide IV so produced was readily purified and completely characterized. This procedure was found to be most applicable to acid chlorides which are at least as reactive as benzoyl chloride, while the pyridine procedure was preferred for less reactive acylating agents.

Although many methods were investigated, cleavage of the imide IV was best accomplished by heating in fresh pyridine at about 50°C for several hours. Although some imide cleavage could be demonstrated during the acylation step in the pyridine procedure, it appeared to be inhibited in some instances by the presence of pyridine hydrochloride. Therefore, the imide IV was frequently isolated and put back into fresh pyridine. The product of imide cleavage was always a mixture of the tetrahydropyranyl derivatives of the desired transacylation product (V), a coumermycin A_1 tetrahydropyranyl fragment (VI), from which one of the PNC-NH-groupings had been cleaved, and ditetrahydropyranylcoumermycin A_1 (II). The mixture could be fractionated at this stage in order to isolate and characterize V and VI (which was done in some cases in order to validate the reaction scheme). In most cases, however, it was found to be more practical to remove the tetrahydropyranyl blocking groups from this mixture of products by exchange with anhydrous methanol at room temperature, catalyzed by *p*-toluenesulfonic acid monohydrate. The second cleavage product then consisted of a mixture of the new antibiotic (VII), PNC-NH-D-OH (VIII), and small amounts of coumermycin A_1 (I). The components were separated from each other by fractional precipitation from ethyl acetate with Skellysolve B, by Craig counter-current distribution, or by column chromatography over silica gel or Sephadex LH20.

The preparation of 3-cinnamamido-4-hydroxy-8-methyl-7-[3-O-(5-methylpyrrolylcarbonyl)noviosyloxy]coumarin (compound 59) and analogs substituted on the aromatic ring or on the vinyl group proceeded readily from the corresponding cinnamoyl chlorides *via* procedures A or B, Table 1. This was as anticipated, since the properties of cinnamic acids parallel closely those of benzoic acids. Therefore no specific examples have been included in the Experimental. It should be noted, however, that the 2- and 4-hydroxy compounds (Nos. 63 and 64) were prepared from 2- and 4-acetoxycinnamoyl chlorides. After the transacylation reaction (at 50°C for 24 hours)

the crude product was subjected to the tetrahydropyranyl cleavage reaction directly. The desired products, isolated by fractional precipitation from ethyl acetate with Skellysolve B, were found to be the hydroxy compounds. It is assumed that cleavage of the acetoxy function occurred simultaneously with cleavage of the tetrahydropyranyl groups.

In all cases the purity of the new antibiotic (VII) was assessed by elemental analyses, infrared and nmr spectra, bioautograph, and/or coumermycin A_1 content by nickel II complex analysis*. Elemental analyses were only moderately sensitive to impurities such as VIII and I, and many samples were found to be discrete hydrates having from 0.5 to 4 moles of H_2O per mole of compound. Hydration was supported by elemental analysis data and independent KARL FISCHER moisture determinations. Infrared spectra were generally very much alike from one compound to another. The nmr spectra, on the other hand, were very informative. The resonances for CH_3O–, two types of CH_3–, H_1 and H_2 of the noviose moiety, and H_{AR} of the coumarin and the new amide R were usually sharp, well separated, and integrated with correct ratios only when the compounds were very pure by other criteria. Bioautographs were run on samples which had been subjected to paper-strip

Table 1. MIC's of PNC-NH-$\overset{O}{\overset{\|}{C}}$-$C_6H_5$ (Compound No. 1) and coumermycin A_1

Organism	Compound No. 1*	Coumermycin A_1*
Diplococcus pneumoniae	1.5	0.16
Streptococcus pyogenes	1.6	0.13
Staphylococcus aureus SMITH	0.25	0.0013
Staphylococcus aureus SMITH plus 50 % human serum	8.8	5.2
Staphylococcus aureus novobiocin resistant	12.5	3.1
Staphylococcus aureus coumermycin A_1 resistant	2.7	0.67
Proteus morganii	>100	4.4
Proteus mirabilis	>100	2.2
E. coli JUHL	>100	10.5
Salmonella enteritidis	>100	3.7
Salmonella typhosa	>100	5.2
Klebsiella pneumoniae	100	1.3
Pseudomonas aeruginosa	>100	12.5

* Geometric mean of five determinations. We thank Drs. D. R. CHISHOLM, M. MISIEK, and K. E. PRICE of the Microbiology Research Department of Bristol Laboratories for the microbiological data in Tables 1, 2 and 3.

* Method developed and analyses carried out by P. MONTELEONE, Chemical Control Department, Bristol Laboratories.

Procedure.

Reagent: 0.001 M $NiSO_4 \cdot 5H_2O$ in DMSO-Dissolve approximately 262 mg of pulverized $NiSO_4 \cdot 5H_2O$ in one liter of spectrograde dimethylsulfoxide by stirring or shaking for approximately 4 hours. Filter through a Whatman #2 paper to obtain a clear solution.

Standard: Dissolve 10.0 mg of coumermycin A_1 in 50 ml of DMSO. Pipet 0.50 ml of this solution into a 10 ml volumetric flask, and dilute to volume with 0.001 M $NiSO_4$ reagent. Measure the absorbance of this solution at 420 mμ in a 1 cm cell using 0.001 M $NiSO_4$ reagent as a blank.

Sample: Weigh 10.0 mg portions of sample into each of two 10 ml volumetric flasks. Dissolve and dilute one to volume with 0.001 M $NiSO_4$ reagent, and the other with DMSO (Sample Blank. For most of the compounds assayed, as for coumermycin A_1 itself, the contribution to the absorbance at 420 mμ by the uncomplexed material was negligible). Measure the absorbance in the same way as the standard.

Calculation: % Apparent coumermycin A_1 =

$$\frac{(\text{mg of coumermycin } A_1 \text{ std.})(\text{Abs. of sample} - \text{Abs. of blank})}{(\text{Abs. of std.})(\text{mg of sample})}$$

Sensitivity: Using the test conditions described above, as little as 0.1 % coumermycin A_1 may be determined.

Table 2. CD_{50}'s[a] of PNC-NH-C(=O)-C_6H_5 (Compound No. 1) and coumermycin A_1

Organism	Route	Compound No. 1	Coumermycin A_1
Staphylococcus aureus SMITH	im	3.3	0.37
	oral	3.7	10.3
Staphylococcus aureus 1633-2	im	9.0	2.5
	oral	44	>400
Streptococcus pyogenes A	im	74	65
	oral	110[b]	>400[b]
Diplococcus pneumoniae	im	54[b]	
	oral	107[b]	>400[b]

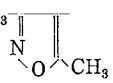
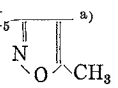
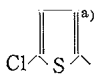
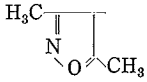
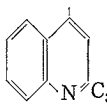
a) Average of three or more determinations.
b) Two treatments with the indicated dose.

Table 3. Semisynthetic coumermycins PNC-NH-C(=O)-R, VII

Compound No.	R	Method [k]	MIC Ratio [g]	dec. pt. °C	% C		% H	
					calc.[h]	found	calc.	found
1	C_6H_5[d] (Na^+ salt)	A	1.0	265	60.59	60.18	5.08	5.06
2	2-$CH_3C_6H_4$	A	1.0	234	63.36	63.44	5.65	5.86
3	3-$CH_3C_6H_4$	A	1.0	195	63.36	63.42	5.65	5.65
4	4-$CH_3C_6H_4$[a]	A	1.0	239	62.42	62.59	5.73	5.77
5	3,5-$(CH_3)_2C_6H_3$	A	0.50	223	63.86	63.53	5.84	6.19
6	2,4,6-$(CH_3)_3C_6H_2$	B	0.50	161	64.34	64.48	6.03	6.45
7	2-$C_6H_5CH_2C_6H_4$[e]	B	1.0	187	62.71	62.85	5.95	5.79
8	1-naphthyl	C	1.0	177	65.41	65.02	5.33	5.59
9	2-$CF_3C_6H_4$	B	1.0	153	58.18	58.07	4.88	5.03
10	3-$CF_3C_6H_4$[c]	B	1.0	141	55.89	55.98	4.98	4.84
11	4-$CF_3C_6H_4$	B	1.0	157	58.18	58.15	4.88	5.19
12	4-$H_2NC_6H_4$	E	0.50	170	61.28	61.53	5.47	5.73
13	3,5-$(H_2N)_2C_6H_3$[a]	E	2.0	175	58.94	58.90	5.59	5.88
14	4-$O_2NC_6H_4$	A	0.25	165	58.40	58.58	4.90	5.45
15	3,5-$(O_2N)_2C_6H_3$	B	>4	169	54.54	54.74	4.43	4.99
16	4-HOC_6H_4	C	8	190	61.18	60.94	5.30	5.39
17	2,4-$(HO)_2C_6H_3$	B	4.0	184	59.61	59.36	5.16	5.62
18	2,6-$(HO)_2C_6H_3$	B	10	172	59.61	59.90	5.17	5.30
19	4-$CH_3OC_6H_5$	A	1.0	170	61.74	61.86	5.51	5.59
20	2,4-$(CH_3O)_2C_6H_3$	B	2.0	140	60.72	61.00	5.56	5.68
21	2,6-$(CH_3O)_2C_6H_3$	B	0.25	144	59.81	59.69	5.61	5.69
22	3,4,5-$(CH_3O)_3C_6H_2$	A	2.0	126	59.04	58.85	5.68	5.77
23	4-$C_6H_5OCH_2CH_2OC_6H_4$	B	0.064	144	64.27	64.35	5.53	5.67
24	2-FC_6H_4	B	4	148	60.98	61.28	5.12	5.46
25	4-FC_6H_4[a]	A	4	110	60.09	60.38	5.21	5.15
26	3-ClC_6H_4	B	2.0	140	59.38	59.36	4.98	5.53
27	4-ClC_6H_4	B	2.0	250	59.38	59.32	4.98	5.33
28	2,5-$Cl_2C_6H_3$[d]	B	1.0	144	55.66	55.74	4.67	5.00
29	2,6-$Cl_2C_6H_3$	B	0.50	161	56.28	56.38	4.57	5.22
30	3,4-$Cl_2C_6H_3$[b]	B	0.25	159	54.79	54.49	4.75	4.84
31	3,5-$Cl_2C_6H_3$	B	2.0	150	56.28	56.81	4.57	4.60

(To be continued)

Table 3 (continued)

Compound No.	R	Method [h]	MIC Ratio [g]	dec. pt. ℃	% C		% H	
					calc.[h]	found	calc.	found
32	4-BrC_6H_4	A	1.0	165	55.44	55.02	4.66	4.65
33	4-IC_6H_4 [a]	A	1.0	125	51.38	51.36	4.43	4.70
34	2-HO-3-$CH_3C_6H_3$ [a]	B	1.0	173	60.84	61.10	5.59	5.84
35	3-HO-4-$CH_3C_6H_3$	B	1.0	180	61.73	61.37	5.50	5.63
36	4-CH_3-3-$NO_2C_6H_3$ [a]	C	0.50	246	58.17	58.21	5.18	5.16
37	3-NH_2-4-$CH_3C_6H_3$	E	1.0	225	i			
38	4-NH_2-3EtC_6H_3	E	0.0016	202	62.35	62.12	5.87	6.29
39	2-HO-3$NO_2C_6H_3$ [f]	D	8.0	>350	50.47	50.48	5.31	5.06
40	4-HO-3-$NO_2C_6H_3$	B	40	162	56.97	57.45	4.78	5.33
41	3-HO-4-$NO_2C_6H_3$ [b]	C	4.0	177	55.43	55.71	4.95	4.90
42	3-NH_2-4-HOC_6H_3 [a]	E	4.0	222	58.85	59.07	5.41	5.94
43	4-NH_2-3-HOC_6H_3 [b]	E	2.0	232	58.03	58.18	5.50	5.86
44	4-HO-3-($Et_2CHCONH$)C_6H_3 [b]	F	8.0	146	60.07	60.39	6.13	6.09
45	4-HO-3-(C_6H_5CONH)C_6H_3 [e]	F	2.0	147	59.06	59.17	5.48	5.55
46	3-CH_3O-2-$NO_2C_6H_3$ [a]	D	8.0	184	56.80	56.90	5.06	5.07
47	3-(nC_4H_9O)-4-$NO_2C_6H_3$ [d]	A	0.12	198	56.36	56.16	5.81	5.78
48	2-HO-4-$EtOC_6H_3$	B	1.0	182	60.73	60.94	5.56	5.78
49	2-EtO-1-naphthyl	B	0.25	175	64.81	64.89	5.44	5.86
50	4-HO-3,5-$I_2C_6H_2$ [j]	A	4.0	204	43.27	45.08	3.51	4.11
51	2,6-$Cl_2C_6H_3$- 	B	2.0	153	56.61	56.64	4.48	4.90
52	C_6H_5-  [a]	B	4.0	122	61.58	61.32	5.32	5.47
53	 [a]	A	0.25	243	54.06	54.24	4.87	4.71
54		A	2.0	258	56.08	56.09	4.87	5.20
55	2-Pyridyl [b]	G	2.0	140	58.91	59.10	5.43	5.30
56		B	0.50	231	58.91	58.94	5.43	5.63
57	2-Quinolyl	C	2.0	155	63.44	63.82	5.17	5.34
58		C	4.0	153	64.80	64.50	5.73	5.91
59	$C_6H_5CH=CH-$	A	1.0	201	64.07	63.69	5.54	5.31
60	4-$(CH_3)_2CHC_6H_4CH=CH-$	B	2.0	163	65.44	65.51	6.10	6.48
61	2-$CH_3OC_6H_4CH=CH-$	A	1.0	180	62.95	63.30	5.59	5.22
62	4-$CH_3OC_6H_4CH=CH-$ [a]	A	0.12	195	62.09	61.95	5.67	5.64
63	2-$HOC_6H_4CH=CH-$ [a]	A	0.50	190	61.57	61.77	5.48	4.93
64	4-$HOC_6H_4CH=CH-$ [a]	A	0.25	196	61.57	61.95	5.48	5.75

(To be continued)

Table 3 (continued)

Compound No.	R	Method [k]	MIC Ratio [g]	dec. pt. °C	% C		% H	
					calc.[h]	found	calc.	found
65	3-$O_2NC_6H_4CH{=}C(Et)$-	B	2.0	150	60.77	61.06	5.39	5.55
66	4-$CH_3OC_6H_4CH{=}C(4\text{-}CH_3OC_6H_4)$-	B	>4	182	65.24	65.31	5.61	5.78
67	$C_6H_5CH{=}CCl$-	B	1.0	196	60.69	60.75	5.09	5.39

a) hemihydrate.
b) monohydrate.
c) sesquihydrate.
d) dihydrate.
e) 2.5 H_2O.
f) tetrahydrate.
g) Minimum inhibitory concentration of compound *vs. Staphylococcus aureus* SMITH in Antibiotic Assay Broth (BBL), pH 6.0, divided by the MIC for Compound 1 in the same medium. Compound 1 had an MIC of from 0.0125 mcg/ml to 0.05 mcg/ml under these conditions.
h) In nearly all cases, nitrogen analyses which were within ±0.3 % of the theoretical values were also obtained.
i) Sample was too small for analysis but was made by reduction of compound 36, which was very pure.
j) Elemental analyses always indicated that *ca.* 10 % of the iodine present in starting acid was lost during reaction.
k) Methods (details in Experimental): A, acid chloride in pyridine at 25° to 50°C, several hours; B, acid chloride in pyridine at reflux, 1~3 hours; C, acid chloride in THF-triethylamine at 0~65°C; D, mixed anhydride in THF-triethylamine at 0~65°C; E, catalytic reduction of one or more nitro groups; F, acylation of compound 42; G, acid chloride in THF-pyridine at 25°C, several hours.

chromatography*, which gave good separations of the new antibiotics VII from coumermycin A_1. Calibration of the paper chromatography-bioautograph system with coumermycin A_1 permitted ready quantitation of 0.5 % or more contamination of the sample by coumermycin A_1 in most cases. Samples were judged to be pure on a bioactivity basis when the coumermycin A_1 content was found to be $\leqq$0.5 % (weight).

All of the compounds VII were found to be active antibiotics. Their structure-activity relationships will be described in detail in a forthcoming publication. The *in vitro* activities of compound No. 1 and coumermycin A_1 against a variety of organisms are compared in Table 1. Table 2 is particularly informative, in that it shows that in spite of coumermycin A_1's consistently superior activity *in vitro*, compound No. 1 has considerably greater therapeutic activity *via* the oral route. This is particularly significant because coumermycin A_1 cannot be employed *via* the intramuscular route because of its irritation liability and poor absorption from the site of injection in higher animals. The physical properties and relative *in vitro* activities of the semisynthetic coumermycins are reported in Table 3.

* Procedure due to C. A. CLARIDGE and V. Z. ROSSOMANO, Microbiology Research Department, Bristol Laboratories. Chromatography was carried out on 1/2" strips of S & S589 Blue Ribbon paper in the solvent system acetone: 0.1 M triethanolamine adjusted to pH 7.0 with glacial acetic acid: 2 : 3. The new antibiotics had Rf's of 0.8~0.9, while coumermycin A_1 had Rf 0.3~0.4. Bioautography was done on agar plates seeded with *Staphylococcus aureus* ATCC 6538P.

Experimental*,†

2′,2′-O,O-Ditetrahydropyranylcoumermycin A_1 (II).

Finely ground coumermycin A_1, 5.5 g was stirred with 50 ml of dihydropyran and a trace of *p*-toluenesulfonic acid monohydrate (2~3 mg) was added. The mixture was stirred in a stoppered flask under anhydrous conditions at 25°C for 3½ hours, dissolution of the solid being complete after 2 hours.

The solution was evaporated to dryness *in vacuo* at minimum temperature (below 40°C) and the residue remaining was dissolved in boiling acetone (30 ml). Hot ethanol was slowly added with stirring and heating until 100 ml had been added. The product crystallized upon cooling overnight to yield 5.3 g (84 %) of fine cube-like crystals. An additional 810 mg (13 %) separated as a second crop from the mother liquor after standing at −5°C for 24 hours.

A sample (1.7 g) was recrystallized twice from acetone-ethanol to give a pure sample (1.5 g), mp (decomposition) above 200°C.

Analysis. Calculated for $C_{65}H_{75}O_{22}N_5$: C 61.06, H 5.91, N 5.47.
Found: C 61.00, H 5.83, N 5.56.

Neutral equivalent: Found 623, calculated 634.

Amberlyst 15 (H^+) resin may be substituted for toluenesulfonic acid as the catalyst in the reaction. In this case, filtration of the catalyst and concentration of the filtrate under anhydrous conditions produces a syrup from which a mixture of mono-, di-, tri-, and tetra-tetrahydropyranyl coumermycin A_1 may be precipitated as an amorphous solid by triturating in the cold with a minimal amount of dry methanol. Evidence that this was indeed a mixture of the named compounds was initially obtained from thin-layer chromatography on silica gel plates, in a solvent system consisting of 9 : 21 : 8 (parts by volume) of methyl acetate : 2-propanol : concentrated ammonium hydroxide. In this system the Rf values are: Coumermycin A_1, 0.3; monotetrahydropyranyl derivative, 0.45~0.50; di-, tri-, and tetra-tetrahydropyranyl derivatives, an elongated zone at Rf 0.60 to 0.70. Individual tlc of the separated products confirmed these Rf values. A CRAIG countercurrent distribution separation was run on a 15-g sample of the mixture using ½ volume upper phase to 1 volume lower phase from a system of 5 : 1 : 5 : 1 of CCl_4 : $CHCl_3$: CH_3OH : H_2O over 1001 transfers, 97.5 % of the solid being recovered in total. The recoveries from the major concentrations, as determined by ultraviolet absorption at 345 mμ, were as follows:

2′,2′,4,4-O,O,O,O-Tetratetrahydropyranylcoumermycin A_1.

The tetra-substituted tetrahydropyranyl ether of coumermycin A_1 was recovered from tubes 21 through 40 as a pure crystalline solid, 3.68 g, mp (decomposition) above 200°C.

Analysis. Calculated for $C_{75}H_{91}N_5O_{24}$: C 62.27, H 6.34, N 4.84.
Found: C 62.03, H 6.31, N 4.94.

2′,2′,4-O,O,O-Tritetrahydropyranylcoumermycin A_1.

The tri-substituted tetrahydropyranyl ether of coumermycin A_1 was recovered from tubes 41-70 as a pure crystalline solid, 3.8 g, mp (decomposition) above 200°C.

Analysis. Calculated for $C_{70}H_{83}N_5O_{23}$: C 61.71, H 6.14, N 5.14.
Found: C 61.65, H 6.19, N 5.34.

2′,2′-O,O-Ditetrahydropyranylcoumermycin A_1.

The disubstituted tetrahydropyranyl ether of coumermycin A_1 was recovered from tubes 71~100 as a pure crystalline solid, 1.8 g, mp (decomposition) above 200°C. The

* The authors are indebted to R. L. DEVAULT, E. R. MAY, C. D. MCDONNELL, and C. L. SWANSON for many of the preparations reported in this paper; to R. M. DOWNING and C. M. KALINOWSKI for elemental analyses; and to D. F. WHITEHEAD and A. L. VULCANO for the infrared and nmr spectra.

† Melting points are not corrected.

product was identical in its physical characteristics to previously characterized material.

2′-O-Monotetrahydropyranylcoumermycin A_1.

The monosubstituted tetrahydropyranyl ether of coumermycin A_1 was recovered from tubes 101~130 as a pure crystalline solid, 1.6 g, mp (decomposition) above 200°C.

Analysis. Calculated for $C_{60}H_{65}N_5O_{21}$: C 60.35, H 5.66, N 5.86.
Found: C 60.42, H 5.81, N 5.83.

Coumermycin A_1 was recovered unreacted from tubes 300~499, 1.5 g, mp (decomposition) 240~245°C.

3-Benzamido-4-hydroxy-8-methyl-7-[3-O-(5-methyl-2-pyrrolylcarbonyl)noviosyloxy] coumarin. (VII, R=C_6H_5, Compound 1). Method A.

Ditetrahydropyranyl coumermycin A_1 (I), 1278 g (1.00 mole), was dissolved in 16.0 liters of anhydrous pyridine. Water, 36.0 g (2.00 moles), was added, followed by 480 ml (4.00 moles) of benzoyl chloride. The reaction mixture was protected from atmospheric moisture and stirred at 50°C for 22 hours. The solution was concentrated *in vacuo* to 4.5 liters and was then poured into 50.0 liters of vigorously agitated ice water. The acidity of the mixture was quickly adjusted to 2.0 with 6 N HCl. The precipitated solid (V, R=C_6H_5, plus VI, plus II, plus D) was removed by filtration, washed thoroughly with cold water, and dried in an oven at 60°C. The dried intermediate was suspended in 16.0 liters of dry methanol, to which 326 g (1.7 mole) of *p*-toluenesulfonic acid monohydrate was added. The mixture was stirred for 15 hours at 25°C, then poured into 50.0 liters of vigorously stirred ice water. The crude product was filtered off, washed with water at pH 5 to 7, and dried in an oven at 60°C. The title compound was purified by fractional precipitation from ethyl acetate by addition of Skellysolve B. The impurities (I and VIII) precipitated first, after which the title compound was obtained as a cream-colored, crystalline solid, mp 230~235°C (d) after softening at *ca.* 190°C. The yield was 425 g.

PNC-NH-D-OH. (VIII, 2 isomers).

Eleven grams of the crude tetrahydropyranyl intermediate V (plus VI, plus II, plus D) was thoroughly mixed with an equal amount of silicic acid (Baker Analyzed, Reagent) and transferred as a slurry in 10 % ethyl acetate - 90% *n*-hexane to the top of a chromatographic column packed with 600 g of silicic acid and wet with the same solvent. The column was eluted successively with 3.00 liters of 10 % ethyl acetate, 46.66 liters of 20 % ethyl acetate, 2.82 liters of 30 % ethyl acetate, 6.35 liters of 40 % ethyl acetate, and 3.56 liters of 60 % ethyl acetate. The latter three eluates were combined and concentrated to dryness, yielding 1.98 g of solid which was stirred with 200 ml of chloroform. The material which failed to dissolve was filtered off and recrystallized from absolute ethanol, 115 mg. Integration of the nmr spectrum revealed the correct ratios of the various types of protons for structure VI.

Analysis. Calculated for $C_{36}H_{41}N_3O_{13}$: C 59.75, H 5.71, N 5.81; mol wt, 723.69.
Found: C 59.18, H 5.77, N 5.75;
mol wt, 742 (osmometric in tetrahydrofuran).

This tetrahydropyranyl intermediate, 100 mg, was dissolved in a mixture of 8.0 ml of methanol and 2.0 ml of tetrahydrofuran, with gentle warming as required to effect solution. A small amount (*ca.* 0.3 g) of Amberlyst-15 resin (H^+) was added and the mixture was stirred at 25°C for 24 hours. The resin was filtered off, washed with a little methanol, and the filtrate was concentrated to dryness *in vacuo*, leaving a powdery, yellow solid. It was dissolved in 50 ml of hot ethyl acetate and diluted with 115 ml of *n*-hexane. After 22 hours at 4°C the tan precipitate was filtered off and dried for analysis, 57 mg, mp 300°C (d).

Analysis. Calculated for $C_{31}H_{33}N_3O_{12}$: C 58.21, H 5.21, N 6.57.
Found: C 58.52, H 5.84, N 6.34.

3-(4-Chlorobenzamido)-4-hydroxy-8-methyl-7-[3-O-(5-methyl-2-pyrrolylcarbonyl) noviosyloxy]coumarin. (Compound 27). Method B.

p-Chlorobenzoyl chloride, 2.74 g (15.6 millimoles) was carefully added at 25°C to a solution of 5.00 g (3.91 millimoles) of ditetrahydropyranyl coumermycin A_1 in 100 ml of freshly-distilled pyridine. The clear solution was refluxed (115°C) for 2.0 hours and poured into 1250 ml of ice-cold 1.2 N HCl. After standing at 4°C for 15 hours, the tan, amorphous solids were filtered off and washed with four 200 ml portions of water. It was dried to give 5.92 g of crude product. Removal of the tetrahydropyranyl moieties was effected by dissolving 5.0 g of the product in 50 ml of THF and 170 ml of methanol, adding 750 mg of *p*-toluenesulfonic acid monohydrate, and stirring at 25°C for 66 hours. The solution was filtered to remove 512 mg of dark, amorphous solid (discarded), the filtrate was diluted with 200 ml of water and concentrated at 40°C *in vacuo* to remove most of the methanol and THF. The pale, golden solid which precipitated was filtered off and dried, 3.25 g. Of this material, 3.0 g was dissolved in 200 ml of chloroform (warmed gently to dissolve). The solution was cooled to *ca.* 25°C and 486 mg of dark, amorphous solid was filtered off. The filtrate was extracted with five 200 ml portions of 5% aqueous sodium bicarbonate, which removed (as shown by tlc) *p*-chlorobenzoic acid, coumermycin A_1, and PNC-NH-D-OH. The chloroform solution was dried over sodium sulfate, filtered, and diluted with two increments of Skellysolve B to produce successively an impure fraction (344 mg) and the desired title compound, 310 mg.

3-(4-Methyl-3-nitrobenzamido)-4-hydroxy-8-methyl-7-[3-O-(5-methyl-2-pyrrolylcarbonyl)noviosyloxy]coumarin. (Compound 36). Method C.

Ditetrahydropyranyl coumermycin A_1 (II), 10.0 g (7.82 millimoles) was dissolved in 100 ml of tetrahydrofuran (THF) at 25°C, and 5.50 ml (3.96 g, 39.1 millimoles) of triethylamine and 0.28 ml (15.6 millimoles) of water were added. To this mixture was added 4.60 ml (6.24 g, 31.3 millimoles) of 4-methyl-3-nitrobenzoyl chloride (Frinton Laboratories) and the solution was heated at reflux (65°C) for 3.5 hours. The solution turned orange during this time. The reaction mixture was cooled at 4°C for 15 hours and filtered to remove triethylamine hydrochloride. The filtrate was poured into 1.0 liter of ice water and the solid which separated was filtered off, washed, and thoroughly dried, 14.20 g of peach-colored solid. The infrared and nmr spectra showed that this intermediate was the intact *bis* imide IV (R=4-methyl-3-nitrophenyl). Cleavages of the imide in warm pyridine (see procedure D) and of the tetrahydropyranyl groups in methanol-acetone with *p*-toluenesulfonic acid catalysis were carried out in the usual manner. The crude mixture of VII, VIII, and I was dissolved in chloroform and exhaustively extracted with 5% aqueous sodium bicarbonate. The chloroform phase was dried, filtered, and the desired product recovered from it by fractional precipitation with Skellysolve B. The yield was 777 mg of white solid, mp 246~248°C (decomp). Its structure was confirmed by infrared and nmr spectra, and by its elemental analyses (See Table 3).

3-(3-Methoxy-2-nitrobenzamido)-4-hydroxy-8-methyl-7-[3-O-(5-methyl-2-pyrrolylcarbonyl)noviosyloxy]coumarin. (Compound 46). Method D.

3-Methoxy-2-nitrobenzoic acid, 24.68 g (125.2 millimoles) was dissolved in 200 ml of tetrahydrofuran, 21.00 ml (15.20 g, 150.2 millimoles) of triethylamine was added, and the mixture was cooled in an ice bath to 0~5°C. Ethyl chloroformate, 13.11 ml (15.0 g, 138 millimoles), was added and the mixture was stirred at 0°C for 45 minutes. The resulting solution of mixed anhydride was carefully added to a pre-cooled solution of 20.00 g (15.65 millimoles) of ditetrahydropyranyl coumermycin A_1 (II) in 400 ml of tetrahydrofuran containing 4.40 ml (31.3 millimoles) of triethylamine. The reaction mixture was stirred at 0~5°C for 1.5 hours, then at room temperature for 20 hours. The insoluble triethylamine hydrochloride was filtered off and the filtrate was concentrated to *ca.* 200 ml *in vacuo*. The deep yellow concentrate was poured into 2.0 liters of ice-cold Skellysolve B.

The yellow precipitate was separated and dried, 47.8 g. The tetrahydropyranyl groups were removed with *p*-toluenesulfonic acid catalysis in methanol-acetone as previously described to yield the coumermycin *bis* imide (confirmed by absence of amide NH bands in the infrared spectrum). The *bis* imide was cleaved to amide by warming in pyridine at 55°C for 48 hours. The pyridine was removed *in vacuo* and the residue was taken up in *ca.* 500 ml of ethyl acetate and extracted 7 times with 150 ml portions of 5 % aqueous sodium bicarbonate. The solvent phase was dried and filtered, and the product was recovered by fractional precipitation with Skellysolve B. The yield of purified title compound was 465 mg.

3-(4-Aminobenzamido)-4-hydroxy-8-methyl-7-[3-O-(5-methyl-2-pyrrolylcarbonyl)noviosyloxy]coumarin. (Compound 12). Method E.

A 6.37-g sample (0.0100 mole) of 3-(4-nitrobenzamido)-4-hydroxy-8-methyl-7-[3-O-(5-methyl-2-pyrrolylcarbonyl)noviosyloxy]coumarin was dissolved in 200 ml of absolute ethanol and 637 mg of 5 % Pd on carbon was added. Reduction was carried out in a 500 ml Parr hydrogenator at an initial pressure of 50 psi for 17.7 hours. The catalyst was filtered off and washed with a small amount of ethanol. The combined filtrates were reduced to dryness *in vacuo* and the gummy solid was dissolved in 100 ml of ethyl acetate. The solution was heated to reflux and 10 ml of Skellysolve B was added. Cooling produced a dark brown precipitate which was filtered off. In this manner an additional seven fractions were obtained, the last of which was a light cream color and weighed 747 mg. Analysis showed it to be pure (see Table 3) and its nmr spectrum was entirely consistent with the expected structure. The infrared spectrum showed the absence of the 1350 cm^{-1} band for $-NO_2$, which was prominent in the starting material.

3-(3-Benzamido-4-hydroxybenzamido)-4-hydroxy-8-methyl-7-[3-O-(5-methyl-2-pyrrolylcarbonyl)noviosyloxy]coumarin. (Compound 45). Method F.

A solution of 150 mg (0.241 millimole) of 3-(3-amino-4-hydroxybenzamido)-4-hydroxy-8-methyl-7-[3-O-(5-methyl-2-pyrrolylcarbonyl)noviosyloxy]coumarin (Compound 42) in 5.0 ml of pyridine was prepared by stirring at 25°C. Benzoic anhydride, 65.4 mg (0.289 millimole), was added and the solution was stirred at 25°C for 17 hours. It was poured into 250 ml of ice water and acidified to pH 1.5 with 6 N hydrochloric acid. The suspension was stirred for 30 minutes, filtered, washed and dried to give 168 mg of crude product. It was dissolved in ethyl acetate, extracted thoroughly with 5 % aqueous sodium bicarbonate solution, and the solvent phase was dried over sodium sulfate. The product was precipitated by the addition of Skellysolve B, 75 mg, mp 147°C (decomp.).

2-Picolinoyl chloride

2-Picolinic acid, 12.3 g (0.100 mole) was heated at reflux with 50 ml of thionyl chloride for 2.0 hours, and the excess reagent was removed under vacuum. The residue was flashed to dryness several times with Skellysolve B in order to remove excess thionyl chloride. The resulting solid was crystallized from a small volume of dry benzene, giving the title compound as dark green crystals. The infrared spectrum showed it to be *ca.* 95 % pure acid chloride.

3-(2-Picolinamido)-4-hydroxy-8-methyl-7-[3-O-(5-methyl-2-pyrrolylcarbonyl)noviosyloxy]coumarin. (Compound 55). Method G.

A solution of 12.8 g (10.0 millimoles) of ditetrahydropyranyl coumermycin A_1 (II) in 320 ml of tetrahydrofuran containing 12.8 ml of dry pyridine was treated successively with 5.60 g (40.0 millimoles) of picolinoyl chloride and 0.36 ml (20 millimoles) of water at 25°C. The solution at this time was bright blue. It was stirred at 25°C for 6.0 hours with no further color change, concentrated to *ca.* 100 ml *in vacuo*, and poured into 1.0 liter of ice water. The pH was adjusted to 4.7 with 6 N hydrochloric acid, and the precipitated solid was collected and dried, 10.8 g. Imide cleavage of the intermediate IV (R=2 Py) was done by dissolving the 10.8 g in 1080 ml of pyridine and heating at 50°C for 19 hours.

The solution was cooled to 25°C and concentrated to *ca.* 350 ml *in vacuo*. It was poured into 3.5 liters of ice water and the pH was adjusted to 5.0 with 6 N hydrochloric acid. The resulting solid was filtered off and dried, giving 10.6 g of intermediate V (R=2-Py). The tetrahydropyranyl group was removed by stirring the 10.6 g of intermediate in a mixture of 70 ml of acetone, 280 ml of methanol, and 2.7 g of *p*-toluenesulfonic acid monohydrate at 25°C for 1.0 hour. The solution was concentrated *in vacuo* to a syrup and poured into *ca.* 200 ml of water. Fractional precipitation of the resulting solid from ether with *n*-hexane yielded the product as the second and third precipitates, combined weight 380 mg. The infrared and nmr spectra of this product were in full agreement with the structure of the title compound.

3-Dibenzoylamino-4-hydroxy-8-methyl-7-[2-O-tetrahydropyranyl-3-O-(5-methyl-2-pyrrolylcarbonyl)noviosyloxy]coumarin.

Ditetrahydropyranyl coumermycin A_1 (II), 1.00 g (0.734 millimole) was dissolved in 20 ml of freshly distilled pyridine. During a five-minute period, 0.85 ml (0.736 millimole) of benzoyl chloride was added, the temperature being maintained at 25°C, and the solution was stirred at 25°C for 24 hours, during which time it turned a deep orange-brown. It was poured into 200 ml of vigorously stirred ice water and the pH was adjusted to 1.5 with 6 N hydrochloric acid (40 ml). The mixture was stirred for 1 hour, the solid was filtered off, washed with water, and dried *in vacuo*. The crude product, 1.47 g, was crystallized from chloroform and Skellysolve B to give 1.20 g of the title compound, mp 128~130°C (decomp.). The infrared spectrum (KBr pellet) showed strong imide carbonyl bands at 1740 and 1710 cm^{-1}, and the absence of amide NH near 1540^{-1}. Integration of the nmr spectrum revealed the expected ratio of aromatic to aliphatic protons.

Analysis. Calculated for $C_{43}H_{44}O_{12}N_3$: C 66.14, H 5.67, N 3.59.
Found: C 66.00, H 5.09, N 3.50.

3-Dibenzoylamino-4-hydroxy-8-methyl-7-[3-O-(5-methyl-2-pyrrolylcarbonyl)noviosyloxy]coumarin.

The tetrahydropyranyl group was cleaved from the preceding compound by toluenesulfonic acid-catalyzed exchange with methanol in a reaction analogous to that used in the preparation of compound 1, Method A. The product (0.60 g) had a decomposition point of 173~175°C and was devoid of antibacterial activity. Its infrared and nmr spectra were entirely consistent with the named structure.

Analysis. Calculated for $C_{38}H_{36}O_{11}N_2$: C 65.51, H 5.21, N 4.02.
Found: C 65.64, H 5.35, N 3.93.

The N-dibenzoylimide was readily cleaved in warm pyridine (50°C, 24 hours) to give a high yield of 3-benzamido-4-hydroxy-8-methyl-7-[3-O-(5-methyl-2-pyrrolylcarbonyl) noviosyloxy]-coumarin, compound 1, which was identical in all respects with previously characterized material.

3-Dibenzoylamino-4-hydroxy-8-methyl-7-[2-O-benzoyl-3-O-(5-methyl-2-pyrrolylcarbonyl)noviosyloxy]coumarin.

When the procedure described above for 3-dibenzoylamino-4-hydroxy-8-methyl-7-[2-O-tetrahydropyranyl-3-O-(5-methyl-2-pyrrolylcarbonyl)-noviosyloxy]coumarin was carried out on coumermycin A_1, I, the title compound was obtained, mp 175~180°C (decomp.). The infrared and nmr spectra were entirely consistent with the named structure, and the compound had no antibacterial activity.

Analysis. Calculated for $C_{45}H_{40}O_{12}N_2$: C 67.49, H 5.04, N 3.50.
Found: C 67.36, H 5.07, N 3.52.

Dibenzoyl coumermycin A_1 *bis* imide, *via* intermediate IV (R=C_6H_5).

Ditetrahydropyranyl coumermycin A_1 (II), 5.00 g (3.91 millimoles) was dissolved in 100 ml of freshly distilled pyridine and the solution was cooled to −5° to −10°C. Benzoyl chloride, 4.60 ml (39.1 millimoles) was carefully added at a rate such that no

temperature rise was observed, and the reaction mixture was stirred under a nitrogen atmosphere for 6 hours at −5°C. The pyridine hydrochloride which precipitated was removed by filtration and the filtrate was poured into 1.0 liter of ice water. The crude product which precipitated was filtered off, washed with ice water and dried *in vacuo*. It was thoroughly washed with carbon tetrachloride and again dried, yield 6.6 g. At this stage, the infrared spectrum showed the absence of amide NH near 1540 cm^{-1} and had strong carbonyl bands at 1680~1750 cm^{-1}, which suggested the imide structure. The presence of the central 3-methylpyrrole-2,4-dicarboxamide moiety was shown by nmr bands at 2.42 ppm and 11.2 ppm (in $CDCl_3$-DMSO) which are characteristic of the D-pyrrole methyl and NH groups respectively. The tetrahydropyranyl blocking groups were removed with *p*-toluenesulfonic acid catalyst in methanol-acetone, as previously described. The resulting product, 660 mg, had mp 182~185°C (decomp.). The *bis* imide formulation was fully supported by its infrared and nmr spectra.

Analysis. Calculated for $C_{69}H_{67}O_{22}N_5 \cdot H_2O$: C 62.01, H 5.20, N 5.24.
Found: C 62.07, H 5.34, N 5.23.

Solvolysis of the above *bis* imide in pyridine at 55°C for 48 hours gave, after recovery of product as described in Method A, the expected compound 1. Comparison of its infrared and nmr spectra, chromatographic behavior, elemental analyses, and bioassay with previously characterized compound 1 showed them to be identical.

Compounds 16, 17, 18, 34, 35, 39, 40, 41, 48, and 50 were all prepared *via* transacylation with the corresponding acetoxybenzoyl chlorides. After imide cleavage in warm pyridine and tetrahydropyranyl cleavage in methanol-acetone with *p*-toluenesulfonic acid, some of the phenolic hydroxyls in each sample remained acetylated. Although these acetoxy compounds had some bioactivity, it was always raised by complete removal of acetoxy groups as follows: The crude cleavage mixture of VII, VIII, and I was dissolved in as much liquid ammonia as required to give a clear solution (frequently *ca.* 10 ml/gram of sample). It was allowed to evaporate at room temperature, and the residue was dissolved in water. On adjustment of the pH to *ca.* 1.5 with dilute hydrochloric acid, the crude product mixture (VII, VIII, and I, now minus all acetoxy groups) precipitated. It was filtered off, dried, and the desired compounds VII were obtained in each case by fractional precipitation from ethyl acetate or chloroform solution with Skellysolve B.

3-(Thiazole-4-carboxamido)-4-hydroxy-8-methyl-7-[3-O-(5-methyl-2-pyrrolylcarbonyl) noviosyloxy]coumarin. (Compound 54).

Thiazole-4-carboxylic acid was converted to the acid chloride by brief reflux with excess thionyl chloride. The excess reagent was completely removed in vacuum, and a solution containing 12.8 g (10.0 millimoles) of ditetrahydropyranyl coumermycin A_1 and 180 mg of water in 200 ml of pyridine was added to 5.88 g (40.0 millimoles) of thiazole-4-carbonyl chloride. The mixture was kept at 50°C for 24 hours, and the crude product was precipitated by adding the solution to 2 liters of ice water and acidifying to pH 4.5 with dilute hydrochloric acid. It was filtered off, dried, and dissolved in a mixture of 200 ml of acetone and 30 ml of methanol containing 600 mg of *p*-toluenesulfonic acid monohydrate. It was agitated for 24 hours, then concentrated to a small volume and diluted with water. The resulting precipitate was dissolved in ethyl acetate, exhaustively extracted with 5 % aqueous sodium bicarbonate, and dried over sodium sulfate. Partially purified product, 5 g, was obained by fractional precipitation with Skellysolve B. Three grams of this product was dissolved in *ca.* 15 ml of 1 : 1 acetone : methanol and fractionated on a 2.5×35 cm column of Sephadex LH20, which was prepared and developed with the same solvent. The peak fractions, as determined by maximal ultraviolet absorption at 365 mμ in ethyl acetate and by maximal antibiotic assay, were combined, flashed dry, and the residue crystallized from ethyl acetate and Skellysolve B. The infrared and nmr spectra substantiated the structure.

Analysis. Calculated for $C_{28}H_{29}O_{10}N_5S$: N 7.01, S 5.35.
Found: N 7.30, S 5.12.

Literature Cited

1) Bell, F.: The migration of acyl groups in *o*-aminophenols. II. J. Chem. Soc. 1931 : 2962～2967, 1931.
2) Cunningham, B. A. & G. L. Schmir: Hydroxyl group participation in amide hydrolysis. The influence of catalysts on the partitioning of a tetrahedral intermediate. J. Am. Chem. Soc. 89 : 917～922, 1967.
3) Hinman, J. W.; H. Hoeksema, E. L. Caron & W. G. Jackson: The partial structure of novobiocin (streptonivicin). II. J. Am. Chem. Soc. 78 : 1072～1074, 1956.
4) Kakwaguchi, H.; M. Okanishi & T. Miyaki: Coumermycin and salts thereof. U. S. Pat. 3,201,386, Aug. 17, 1965.
5) Kawaguchi, H.; H. Tsukiura, M. Okanishi, T. Miyaki, T. Ohmori, K. Fujisawa & H. Koshiyama: Studies on coumermycin, a new antibiotic. I. Production, isolation and characterization of coumermycin A_1. J. Antibiotics, Ser. A 18 : 1～10, 1965.