highest dose tested of 100 mg/kg. Additional *in vivo* data can be found in Table II.

TABLE II

Penicillin	Approx single sc dose[a] ED50, mg/kg *S. aureus* UC-76	ED50, mg/kg *S. aureus* H-228	Resistance index in mice[b]
Penicillin G	1	80	80
Oxacillin	16	25	1.6
2,4,6-Trimethylcycloheptatrienecarbonyl penicillin	70	450	6.4

[a] Standard mouse protection tests [M. W. Fisher, M. C. Manning, L. A. Gagliardi, M. R. Gaetz, and A. L. Erlandson, *Antibiot. Ann.*, 293 (1959–1960)] involving single subcutaneous dose therapy (0.5 ml) concurrent with lethal intraperitoneal mucinized challenges with either *S. aureus* UC-76 (sensitive) or H-228 (resistant); groups of ten mice were used. [b] *In vivo* resistance index obtained by comparing data from the two *S. aureus* strains.

Experimental Section

Tlc was carried out on silica gel HF plates in the solvent system BuOH–H_2O–AcOH (80:80:2) with 6-aminopenicillanic acid (Aldrich Chemical Co.) as standard. The spots were detected under uv light and with the alkaline NH_2OH–$FeCl_3$ spray.

General Procedure for the Preparation of Penicillins.—The particular cycloheptatrienecarboxylic acid was refluxed in Et_2O in the presence of excess $SOCl_2$ for 2–3 hr. Evaporation of the solution and removal of excess reagent gave the corresponding acid chloride which was examined by ir spectroscopy for the absence of C=O peaks. The preparation of the penicillins followed the procedure described below.

A solution containing 0.39 g (0.002 mole) of 2,4,6-trimethylcycloheptatrienecarbonyl chloride[6] in 20 ml of CH_2Cl_2 was added dropwise to a stirred solution of 6-aminopenicillanic acid (0.42 g, 0.002 mole) and Et_3N (0.7 ml) in 7 ml of CH_2Cl_2 at 0°. The solution was stirred for 2 hr after the addition was completed and then allowed to warm to room temperature. Evaporation to dryness followed by addition of Me_2CO (20 ml) and filtration afforded a pale yellow solution which was concentrated to dryness. The residue was dissolved in 15 ml of H_2O, the solution was covered with 30 ml of EtOAc, and the pH was adjusted to 2.2 with 10% aqueous H_2SO_4 at 0°. The organic phase was separated, washed rapidly (H_2O), dried (Na_2SO_4), and filtered. A solution of 50% potassium 2-ethylhexanoate in BuOH was added to the filtrate and the whole was concentrated to a small volume (*ca.* 5 ml). Et_2O was then added to the neutral solution until precipitation was complete. After standing overnight at 0°, the precipitated K salt of the penicillin was filtered, washed (Et_2O), and dried (P_2O_5); yield, 0.3 g of an almost colorless solid. Tlc revealed the presence of only one spot. *Anal.* ($C_{19}H_{22}KN_2O_4 \cdot 2H_2O$) C, 50.08; H, 4.90; N, 6.23; S, 7.12. Found: C, 50.62; H, 4.60; N, 5.93; S, 6.72.

Acknowledgment.—The authors thank Dr. C. L. Heifetz of these laboratories for carrying out the biological tests.

Synthetic Penicillins Derived from Benznorcaradienecarboxylic Acids

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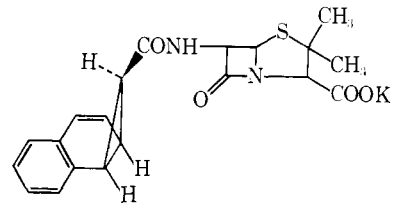
The enhanced antibacterial activity of certain commercially available penicillins against penicillinase-producing strains of *Staphylococcus aureus* has been attributed[2] to a "steric effect" exerted by the bulky group(s) attached to the acyl portion. Thus, in some penicillins[3] containing *ortho*-substituted aromatic acyl groups, such as methicillin, diphenicillin, nafcillin, etc., the cleavage of the lactam ring by penicillinase-producing bacteria is considerably retarded or even prevented, presumably due to the inability of the active sites of the enzyme to achieve the prerequisite fit with the specific groups on the penicillin.

We wish to describe the preparation of a series of novel semisynthetic penicillins **1**, derived from benznorcaradienecarboxylic acids (1a,7b-dihydro-1H-cyclopropa[*a*]naphthalene-1-carboxylic acids). From con-



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siderations of molecular models of these derivatives, it was anticipated that the unique spacial arrangement[4] of the acyl group in the vicinity of the lactam ring would confer some interesting antibacterial properties, particularly against penicillinase-producing strains of *S. aureus*.

The majority of the benznorcaradienecarboxylic acids required for this study were prepared by published procedures. The penicillins were prepared by the acid chloride method and were isolated in the form of amorphous potassium salts. Their homogeneity was established qualitatively by tlc and ir spectral data, and quantitatively by a uv spectral assay and colorimetric procedures.

Results of the antibacterial tests[5] are listed in Tables I and II. It can be seen that, in general, the derivatives exhibit *in vitro* antibacterial activities against penicillinase-producing strains, which are somewhat superior to penicillin G, but none approach the levels shown by oxacillin. The most promising compound appeared to be the unsubstituted penicillin **1**, which has a favorable ratio of activities *vs.* the two *S. aureus* strains. It is of interest to note that minor variations in the nature and positions of substituents in the substituted compound caused appreciable variations in *in vitro* activity, particularly against *Streptococcus pyogenes* (**15** and **16**). Compound **1** was well tolerated in mice at the highest dose tested of 100 mg/kg. Additional data on *in vivo* tests using **1** are provided in Table II, together with comparative data on penicillin G and oxacillin.

Experimental Section

Melting points are uncorrected. Tlc were developed in the solvent system BuOH–H_2O–AcOH (80:80:2) using silica gel HF plates and spots were detected by uv visualization and the alkaline NH_2OH–$FeCl_3$ spray.

1a,2,3,7b-Tetrahydro-2,3-dichloro-1H-cyclopropa[*a*]naphthalene-1-carboxylic Acid (13).—A solution containing 1.86 g (0.01 mole) of 1a,7b-dihydro-1H-cyclopropa[*a*]naphthalene-1-

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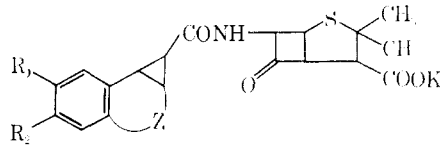
(2) R. Knox, *Nature*, **192**, 492 (1961).

(3) For a review see, J. O. Klein and M. Finland, *New Engl. J. Med.*, **269**, 1019, 1074, 1129 (1963).

(4) R. Huisgen and G. Juppe, *Chem. Ber.*, **94**, 2332 (1961).

(5) M. W. Fisher, M. C. Manning, L. A. Gagliardi, M. R. Gaetz, and A. L. Erlandson, *Antibiot. Ann.*, 293 (1959–1960).

TABLE I



Penicillin	Z	Purity[a]	R_1	R_2	Min inhib concn, μg/ml[b] *S. aureus* UC-76[c]	*S. aureus* TU-12404[c]	*Strep. pyogenes*	Ref[d]
1		95	H	H	6.3	12.5	0.4	4
2		93	H	H	0.8	12.5	0.2	*f*
3		89	H	H	0.4	12.5	0.1	*f*
4		90	Me	H	0.8	25.0	0.4	6
5		90	Me	H	0.4	12.5	0.1	
6		88	Me	Me	1.6	25.0	0.8	*f*
7		93	Me	Me	0.8	50.0	0.2	
8		83	H	H	0.8	50.0	0.2	4
9		77	H	H	0.8	12.5	0.4	4
10		79	H	H	0.8	12.5	0.4	*f*
11		86	Me	H	1.6	12.5	0.4	*f*
12		91	Me	Me	3.1	25.0	0.8	*f*
13		88	H	H	0.4	12.5	0.1	
14		89	H	H	1.6	12.5	0.2	
15		87	H	H	0.4	12.5	0.025	
16[e]		83	H	H	1.6	25.0	0.0125	
Penicillin G					0.0125	50.0	0.0063	
Methicillin					0.8	3.1	0.1	
Oxacillin					0.1	0.4	0.025	

[a] Determined by the NH_2OH colorimetric procedure [J. H. Ford, *Anal. Chem.*, **19**, 1004 (1947)] or by a uv assay. The latter was estimated by comparing the extinction coefficients of the pure benznorcaradienecarboxylic acid derivative and the penicillin derived from it. Compounds **1**–**16** were homogeneous by tlc. [b] Measured in broth by serial twofold dilutions. The tubes were examined macroscopically for end-point determinations after incubation for 18 hr at 37°. For details, see ref 5. [c] *S. aureus* UC-76 and TU-12404 refer to penicillinase nonproducers and producers, respectively. [d] The reference indicates the preparation of the benznorcaradienecarboxylic acid or its substituted analogs. [e] This compound was prepared from penicillin **15** by catalytic hydrogenation in aqueous methanol (10% Pd–$BaSO_4$) at 3.5 kg/cm². [f] G. Juppe and R. Huisgen, *Ann.*, **646**, 1 (1961).

carboxylic acid[4] in 30 ml of $CHCl_3$ was saturated with Cl_2 at 0°. After stirring at room temperature for 30 min, the solvent and excess Cl_2 were removed under reduced pressure leaving a white solid (2 g). Recrystallization (MeOH) gave the pure product, mp 151–152°. *Anal.* ($C_{12}H_{10}Cl_2O_2$) Cl.

1a,2,3,7b-Tetrahydro-2,6-dimethyl-1H-cyclopropa[*a*]naphthalene-1-carboxylic Acid (5).—A solution of 1a,7b-dihydro-2,6-dimethyl-1H-cyclopropa[*a*]naphthalenecarboxylic acid[6] (0.635 g, 3 mmoles) in 75 ml of MeOH was hydrogenated (5% Pd–C) at 3.5 kg/cm². After 24 hr, the catalyst was filtered and the filtrate was evaporated to give the product as a white solid. Recrystallization (MeOH) gave the pure product (0.13 g), mp 168–171°. *Anal.* ($C_{14}H_{14}O_2$) C, H.

(6) R. Huisgen and G. Juppe, *Tetrahedron*, **15**, 7 (1961).

TABLE II

Penicillin	Acute single sc dose[a] ED_{50}, mg/kg: *S. aureus* UC-76	*S. aureus* H-228[b]	Resistance index[c]
Penicillin G	1	80	80
Oxacillin	16	25	1.6
Compound **1**	20	110	5.5

[a] Standard mouse protection tests, involving single-dose therapy concurrent with lethal intraperitoneal challenges. Groups of ten mice were used. For details, see ref 5. [b] *S. aureus* H-228 refers to a penicillinase-producing strain. [c] *In vivo* resistance index was obtained by comparing data from the two *S. aureus* strains.

3-Azido-1a,2,3,7b-tetrahydro-2-hydroxy-1H-cyclopropa[*a*]-naphthalene-1-carboxylic Acid (14).—A solution of 2,3-epoxy-1a,2,3,7b-tetrahydro-1H-cyclopropa[*a*]naphthalene-1-carboxyl ethyl ester[6] (2.3 g, 0.01 mole) in 40 ml of methyl Cellosolve containing 1.3 g of NaN_3, 0.5 g of NH_4Cl, and 2 drops of H_2O was refluxed for 4 hr. After cooling and evaporating to dryness, the residue was extracted (Et_2O) to give 0.9 g of a pale yellow liquid, exhibiting strong N_3 and OH absorption bands in the ir spectrum. A portion of the ester (0.18 g) was dissolved in MeOH containing 10% NaOH and the solution was allowed to stir at 0° for 1 hr. The MeOH was removed under reduced pressure without heating, the residue was dissolved in H_2O and acidified, and the solution was extracted (Et_2O). Processing the extracts in the usual way afforded the product as an amorphous white powder, mp 115–120°. *Anal.* ($C_{12}H_{11}N_3O_3$) C, H, N.

3-Azido-1a,2,3,7b-tetrahydro-2-methoxy-1H-cyclopropa[*a*]-naphthalene-1-carboxylic Acid (15).—To a refluxing solution of the preceding compound (2.73 g, 0.01 mole) in 30 ml of MeI was added 15 g (0.06 mole) of Ag_2O, in portions. The mixture was stirred under reflux overnight and filtered, and the filtrate was evaporated to dryness (yield 2.5 g). A portion of the oily residue (2.1 g) in 15 ml of MeOH was added for 20 ml of cold MeOH containing 0.56 g of NaOH. After stirring for 30 min at 0°, the solution was concentrated at low temperature, the residue was acidified with dilute HCl, and the solution was extracted (Et_2O). Processing the Et_2O extracts afforded the product as a pale yellow oil which was used as such in further steps; ir (liquid film), 2100 cm^{-1} (azide).

General Procedure for the Preparation of Penicillins.—The particular acid was converted to the acid chloride by refluxing in C_6H_6 containing a slight excess of $SOCl_2$ during 2–3 hr. The acid chlorides obtained by removing the solvent and drying the residue over KOH pellets *in vacuo* had the expected spectral properties and were used as such. A typical preparation is described in detail in the case of **1**.

A solution of 1a,7b-dihydro-1H-cyclopropa[*a*]naphthalene-1-carbonyl chloride[4] (0.4 g, 0.2 mmole) in 15 ml of CH_2Cl_2 was added dropwise to a stirring solution of 6-aminopenicillanic acid[7] (0.42 g, 2 mmoles) and Et_3N (0.7 ml, 0.46 mmole) in $CHCl_3$ (7 ml) at 0°. The mixture was stirred at 0° for 2 hr and warmed to room temperature, and the solvent was removed under reduced pressure. Me_2CO (20 ml) was added, the insoluble material was filtered, the filtrate was concentrated, and the residue was dissolved in H_2O (15 ml). The solution was covered with 20 ml of EtOAc and cooled and the pH was adjusted to 2.2 with cold aqueous H_2SO_4. The organic layer was separated, washed quickly (H_2O), dried (Na_2SO_4), and heated with 1 ml of a 50% solution of potassium 2-ethylhexanoate in BuOH. The solution was concentrated at low temperature to about 5 ml and heated with Et_2O until precipitation was complete. After standing at 5° for several hours, the precipitate was filtered, washed well (Et_2O), and dried; yield 0.2 g of an off-white solid. The product exhibited a single spot on tlc.

Anal. Calcd for $C_{26}H_{19}O_4N_2SK \cdot H_2O$: C, 55.00; H, 4.35; N, 6.37; S, 7.26. Found: C, 55.58; H, 4.54; N, 6.90; S, 7.31.

Acknowledgment.—The authors wish to thank Dr. C. L. Heifetz of these laboratories for carrying out the biological tests.

(7) F. R. Batchelor, F. P. Doyle, J. H. C. Nayler, and G. N. Robinson, *Nature*, **183**, 257 (1959).

Researches in the Field of Antiviral Compounds. Mannich Bases of 3-Hydroxycoumarin

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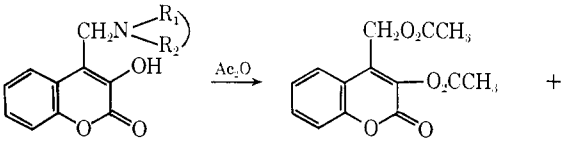
The synthesis of some 2,4-dioxo-3-hydroxyiminochromane derivatives showing good antiviral activity has been dealt with in a previous note;[2] these compounds, however, developed a high degree of cytotoxicity. In order to lower their toxicity we have started synthetic work with the aim of modifying the active molecule while preserving the cyclic α-dicarbonyl structure.

The present note deals with the preparation of several 4-N,N-dialkylaminomethyl-3-hydroxycoumarins obtained through the Mannich reaction. 3-Hydroxycoumarin was treated with formaldehyde and various primary and secondary amines so that products containing an α-dicarbonyl as well as a basic group were obtained; the latter is reported to be present in many antiviral compounds.[3]

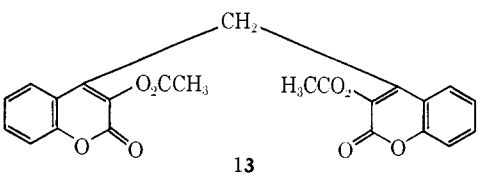
The synthetic steps leading to these compounds are described in the Experimental Section; the synthesized compounds are listed in Table I.

Compounds **3**, **4**, **7**, and **8** are quite unstable Mannich bases and give, when boiled in EtOH, 4,4′-methylenebis-(3-hydroxycoumarin)[4] thus showing that the aminomethyl group replaces position 4.

All the bases react with Ac_2O as reported in the literature for analogous Mannich bases;[5] in fact **12** is obtained by substitution of the aminomethyl group by an acetoxymethyl group and simultaneous acetylation of the 3-hydroxyl group. A by-product [4,4′-methylenebis-(3-acetoxycoumarin) (**13**)] precipitates, its amount depending on the relative instability to heat of the starting base.



1–11 12



13

In order to confirm its structure, **13** was also synthesized by reaction of 4,4′-methylenebis(3-hydroxycoumarin) with Ac_2O. The structure of **12** has been

(1) M. Pigini thanks the National Research Council of Italy for a fellowship;
(2) M. Giannella and F. Gualtieri, *Farmaco, Ed. Sci.*, **23**, 1104 (1968).
(3) (a) R. C. Fort, Jr., and P. Von R. Schleyer, *Chem. Rev.*, **64**, 277 (1964). (b) A. C. Hollinshead and P. K. Smith, *J. Pharmacol. Exp. Ther.*, **123**, 54 (1968); (c) B. Melander, *Antibiot. Chemotherapy*, **10**, 34 (1960).
(4) K. N. Trivedi and S. Sethna, *J. Org. Chem.*, **25**, 1817 (1960).
(5) H. A. Bruson and C. W. MacMullen, *J. Amer. Chem. Soc.*, **63**, 270 (1941).