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 $CHCl_2$ (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.

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

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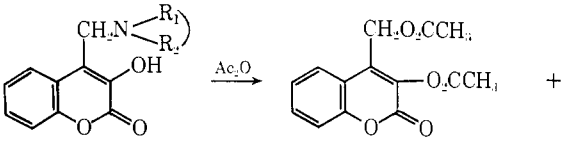
The synthesis of some 2,4-dioxo-3-hydroxyiminochromane derivatives showing good antiviral activity has been dealt with in a previous note;[2] these compounds, however, developed a high degree of cytotoxicity. In order to lower their toxicity we have started synthetic work with the aim of modifying the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

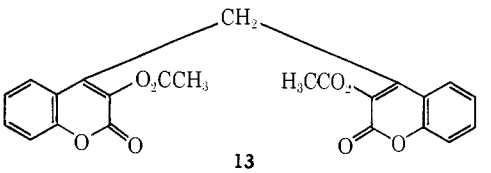
The synthetic steps leading to these compounds are described in the Experimental Section; the synthesized compounds are listed in Table I.

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

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TABLE 1
4-N,N-DIALKYLAMINOMETHYL-3-HYDROXYCOUMARIN AND DERIVATIVES

No.	R_1	R_2	Salts	Mp, °C[a]	Yield, % purified	Formula[b]
2	H	n-C_6H_{13}		78–80	37	$C_{16}H_{23}NO_3$
			Picrate	165–167		$C_{16}H_{23}NO_3 \cdot C_6H_3N_3O_7$
3	CH_3	CH_3		107–110	35	$C_{12}H_{13}NO_3$
			HCl	170–172		$C_{12}H_{13}NO_3 \cdot HCl$
4	C_2H_5	C_2H_5		69–71	30	$C_{14}H_{17}NO_3$
			HCl	122–125		$C_{14}H_{17}NO_3 \cdot HCl$
			Picrate	108–110		$C_{14}H_{17}NO_3 \cdot C_6H_3N_3O_7$
5	CH_2CH_2OH	CH_2CH_2OH		99–100	57	$C_{14}H_{17}NO_5$
			HCl	139–141		$C_{14}H_{17}NO_5 \cdot HCl$
			Picrate	130–132		$C_{14}H_{17}NO_5 \cdot C_6H_3N_3O_7$
6	$CH_2C_6H_5$	$CH_2C_6H_5$		137–140	53	$C_{24}H_{21}NO_3$
			HCl	225–230		$C_{24}H_{21}NO_3 \cdot HCl$
7[c]	C_2H_5	C_6H_5		159–162	31	$C_{18}H_{17}NO_3$
8[c]	CH_2CH_2Cl	CH_2CH_2Cl		139–141	33	$C_{16}H_{15}NO_3Cl_2$
9				100–101	50	$C_{14}H_{15}NO_3$
			HCl	175–177		$C_{14}H_{15}NO_3 \cdot HCl$
			Picrate	155–157		$C_{14}H_{15}NO_3 \cdot C_6H_3N_3O_7$
10				149–151	48	$C_{15}H_{17}NO_3$
			HCl	173–175		$C_{15}H_{17}NO_3 \cdot HCl$
11				138–139	65	$C_{14}H_{15}NO_4$
			HCl	155–157		$C_{14}H_{15}NO_4 \cdot HCl$
			Picrate	175–177		$C_{19}H_{15}NO_4 \cdot C_6H_3N_3O_7$

[a] All compounds melted with decomposition. [b] All compounds were analyzed for C, H, N. [c] No derivatives were made because this compound is not stable in acidic medium.

confirmed by its nmr spectrum (see Experimental Section).

Ir spectra show that the free bases exist, at least in part, as intramolecular dipolar ions, and this precludes the preparation of derivatives at the 3-carbonyl group. Even under more severe conditions, as prolonged boiling, these compounds, as well as their hydrochlorides, give 4,4′-methylenebis(3-hydroxycoumarin) only.

Preliminary tests on some of the synthesized compounds (**6–9**) did not show appreciable antiviral activity.[6] All compounds showed some activity against bacteria at 100–150 μg/ml, and against fungi at 50–100 μg/ml.[7]

Experimental Section

Melting points were determined in capillary tubes on a Büchi apparatus and are uncorrected. The ir spectra were recorded with a Unicam SP 200 spectrometer and the uv spectra (EtOH) with a Unicam SP 800 spectrometer; the nmr spectrum of **12** was recorded by a Varian 60 spectrometer. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values.

4-N-Cyclohexylaminomethyl-3-hydroxycoumarin (**1**).—An EtOH solution of cyclohexylamine (0.015 mole) with 1 ml (0.015 mole) of 40% HCHO was added to a solution of 2 g of 3-hydroxycoumarin[4] (0.012 mole) with stirring and cooling between 0 and 5°. The reaction mixture was then left for 1 day at room temperature. The precipitate was collected and crystallized from EtOH removing the insoluble 4,4′-methylenebis(3-hydroxycoumarin). After recrystallization, the yield was 1.75 g (53%); mp 157–159° dec; ir absorption (Nujol) (cm^{-1}), 1670 (CO), 3100 (OH), broad salt-like band between 2000 and 2800; uv, λ_{max}^{EtOH} 328.0 mμ (log ε 4.18). *Anal.* ($C_{16}H_{19}NO_3$) C, H, N.

The hydrochloride, recrystallized from absolute EtOH, melted at 204–205° dec. *Anal.* ($C_{16}H_{19}NO_3 \cdot HCl$) C, H, N.

The picrate (from EtOH), melted at 172–174° dec. *Anal.* ($C_{16}H_{19}NO_3 \cdot C_6H_3N_3O_7$) C, H, N.

All the compounds, listed in Table 1, were obtained by a similar procedure and recrystallized from the same solvents. Their ir and uv spectra are consistent with the supposed structure.

When the bases **3**, **4**, **7**, and **8** were boiled for 2 hr in EtOH, 4,4′-methylenebis(3-hydroxycoumarin) was obtained. Its properties were identical with that of an authentic sample prepared according to Trivedi.[4]

3-Acetoxy-4-acetoxymethylcoumarin (**12**).—A mixture of a base (1 g) and Ac_2O (5 ml) was refluxed for about 3 hr. Then the solution was spin-evaporated *in vacuo* and the residue was washed (Et_2O). After drying the Et_2O solution (Na_2SO_4), removal of the solvent *in vacuo* gave a solid (**12**), mp 119–120° (from EtOH). The yields vary from 40 to 70% according to the stability of the bases used; ir absorptions (Nujol) (cm^{-1}), 1711 (broad) and 1716 (sharp); uv, λ_{max}^{EtOH} 279.0 mμ (log ε 4.10), 315.0 mμ (shoulder); nmr (DMSO), δ 2.05 and 2.37 (s, CH_3), 5.36 (s, CH_2), and 7.30–8.00 ppm (m, aromatic). *Anal.* ($C_{14}H_{12}O_6$), C, H.

The residue from the ethereal washings was recrystallized from DMF–H_2O. Mixture melting point with 4,4′-methylenebis(3-acetoxycoumarin), obtained as described below, was not depressed.

4,4′-Methylenebis(3-acetoxycoumarin) (**13**).—A mixture of 4,4′-methylenebis(3-hydroxycoumarin) (1 g) and Ac_2O (5 ml) was refluxed for about 2 hr. After cooling, a solid was obtained which recrystallized from DMF–H_2O; yield 85%; mp 245–246° dec; ir absorption (Nujol) (cm^{-1}), 1725 (broad) and 1760 (sharp); uv, λ_{max}^{EtOH} 276.5 mμ (log ε 4.31), 315.0 mμ (shoulder). *Anal.* ($C_{23}H_{16}O_8$) C, H.

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(6) The tests were carried out by the Bristol Co., Syracuse, N. Y., on various strains of viruses, among which were the influenza, vaccinia, and herpes simplex viruses.

(7) The bacteria used were *Salmonella typhi*, *Salmonella paratyphi A and B*, *Staphylococcus aureus*, *Bacillus subtilis*, *Proteus ozk*, *Escherichia coli*, and *Sarcina lutea*. The fungi used were *Fusarium oxysporum lycopersici*, *Fusarium lycopersici*, *Streptomyces griseus*, and *Penicillum sp.*