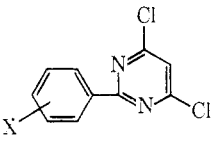
TABLE II



Compd	X	Mp, °C	Yield, %	Formula[a]
IIe	3-Cl	118.5–119	88	$C_{10}H_5Cl_3N_2$
IIe	3,4-Cl_2	122–124	90	$C_{10}H_4Cl_4N_2$
IIf	4-Me	84–85	75	$C_{11}H_8Cl_2N_2$

[a] All compounds were analyzed for C, H, Cl, N.

crystallized on standing. This material was collected on a filter and washed (H_2O) several times to give 8 g (95%) of product, mp 78–82°. Two recrystallizations from heptane afforded an analytical sample (Table I).

Acknowledgment.—The authors are indebted to Messrs. W. B. Edwards III and R. A. Fieber for their technical assistance and to Dr. Sanford B. Rosenman for the antiamoebic assays.

O-Demethyldecarbamoylnovobiocin

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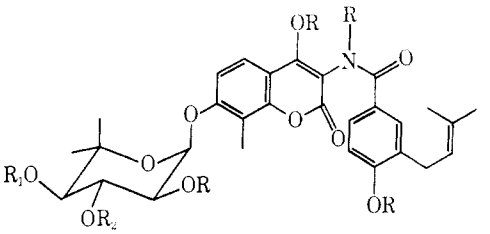
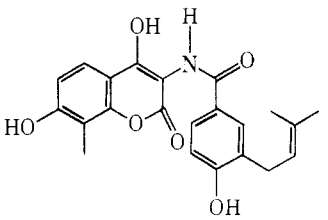
Novobiocin[1] (I), a crystalline antibiotic produced by *Streptomyces niveus*, is of commercial importance. One of the major by-products from the production of I is a biologically inactive material designated compound X. This compound has a uv spectrum identical with I but it has more polar chromatographic characteristics. Because of its apparent similarity and its relevance to the biosynthesis of novobiocin,[2] its structure was determined and is reported in this note.

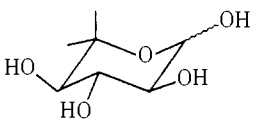
Novobiocin (I) can be isolated by extraction of the filtered fermentation broth with BuOAc. The antibiotic is then extracted into an aqueous buffer at a high pH. This solution is acidified and back-extracted into BuOAc. In this procedure compound X is accumulated in the spent aqueous fraction. It was extracted with EtOAc. The crude material obtained after removal of EtOAc showed five spots on paper chromatography, decarbamoylnovobiocin (II), isonovobiocin, compound X, and a more polar component, in order of decreasing R_f. Removal of the carbamoyl group by treatment with alkali converted this material into a mixture of two compounds, decarbamoylnovobiocin and compound X (spot position unchanged by the saponification). Compound X was obtained pure by countercurrent distribution and by silica gel chromatography. It was crystallized to give material of mp 124–126°; uv spectrum (EtOH), 207 mμ (log ε 4.73), 332 mμ (log ε 4.39); ir, 1685 cm^{-1} (amide C=O), no urethan C=O; nmr (DMF), τ 8.67 and 8.82 (6 H, $>CMe_2$), 8.23 (6 H, CMe_2), 7.71 (3 H, aryl Me), and no OMe signal. *Anal.* Calcd for $C_{29}H_{33}NO_{10}$: C, 62.69; H, 5.99; N, 2.52. Found: C, 62.67; H, 6.12; N, 2.47.



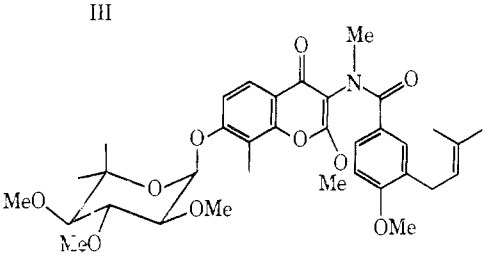
	R_1	R_2	R
I	Me	H_2NCO	H
II	Me	H	H
VI	Me	Me	Me
VII	H	H	H



III



IV



V

These data show that compound X does not contain a carbamoyl group or an O-methyl group. As a working hypothesis we assumed that the sugar moieties of compound X and II were identical except that X contained an OH group where II contained a CH_3O group. The chemical conversions described below of compound X showed this to be true.

Compound X was degraded following procedures[1] used in the structural elucidation of I. Acidic treatment gave two products: a crystalline aglycon, identical by ir spectroscopy with authentic novobiocic acid (III), a known degradation product of I, and an oily ethyl glycoside. This glycoside was shown to contain a *gem*-dimethyl group; by oxidation with H_2CrO_4 it yielded acetone collected as its 2,4-DNP. The glycoside was acetylated to give an oily triacetate verified by nmr. *Anal.* Calcd for $C_{15}H_{24}O_8$: C, 54.21; H, 7.28. Found: C, 54.26; H, 7.46. Further acid treatment of the glycoside yielded a free sugar which reacted with 3.3 equiv of IO_4^-, thereby indicating four vicinal OH groups. These data are consistent with noviose (IV), the sugar moiety which is present in II.

To prove that the configuration of the sugar moiety of X is identical with that of I, both X and II were converted separately to identical permethylated derivatives. Treatment of II with excess CH_2N_2 gave a mixture of two major products. Conversion of this mixture to two pentamethyl derivatives was accomplished by treatment with NaH–MeI in DMF at room temperature. The two major components were separated by alumina chromatography to give a 16% yield of crystalline material, mp 149–151°, and a 37% yield of noncrystalline material. Nmr, ir, and uv spectra support

(1) J. W. Hinman, E. L. Caron, and H. Hoeksema, *J. Am. Chem. Soc.*, **79**, 3789 (1957).

(2) L. A. Kominek in "Antibiotics," Vol. II, D. Gottlieb and P. D. Shaw, Ed., Springer-Verlag, New York, N. Y., 1967, p 253.

structure V for the crystalline material. The noncrystalline material was tentatively assigned VI on the basis of its uv and nmr spectra but its elemental analysis was poor. *Anal.* Calcd for $C_{35}H_{45}O_{10}N$: C, 65.71; H, 7.09; N, 2.19. Found: C, 64.31; H, 7.33; N, 1.88.

In a similar manner X was treated with CH_2N_2 and the crude product was treated with NaH–MeI. Again two major products were formed and these were separated and purified by alumina chromatography to give an 11% yield of a crystalline material and a 39% yield of a noncrystalline material. The two crystalline compounds obtained from II and X were identical in every respect including elemental analyses (*Anal.* Calcd for $C_{35}H_{45}O_{10}N$: C, 65.71; H, 7.09; N, 2.19. Found: C, 66.04; H, 7.16; N, 2.44), mixture melting point, and ir and nmr spectra; the two noncrystalline compounds were identical by ir spectra.

Since both X and II are converted to identical derivatives the stereochemistry of the sugar moieties of the two compounds must be identical and X must be O-demethyldecarbamoylnovobiocin (VII).

Synthesis of 5′-Substituted Derivatives of Inosine[1a]

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Growth inhibitory effects are shown by a variety of adenosine analogs in which either the ribose portion or the purine ring skeleton is modified.[2] The inhibition appears in many instances to be related to the tendency for the adeonosine analog to become converted *in vivo* to a nucleoside 5′-phosphate by adenosine kinase action.[2-4] In addition to adenosine derivatives two inosine derivatives, 7-deaza-[5] and 8-aza-9-deazainosines[6] (the 6-hydroxypurine analogs of tubercidin and formycin, respectively), are also inhibitory to mammalian and bacterial systems and it has been suggested[2] that these effects may be associated with enzymatic conversion of these analogs to their ribonucleotides. Support for this possibility is provided by recent evidence[7,8] for the existence in mammalian cells of inosine kinase. The present report describes the synthesis of some 5′-substituted inosine derivatives, including 5′-amino- and 5′-mercapto-5′-deoxyinosines (**4** and **8**, respectively) which are potentially capable of becoming phosphorylated at the 5′ substituent by an inosine kinase and which might, therefore, exert growth inhibitory effects.

Treatment of 5′-O-(*p*-tolylsulfonyl)-2′,3′-O-isopropylideneinosine (**1**) with sodium azide in dimethyl sulfoxide furnished the 5′-azido nucleoside **2** in good yield (Scheme I); a minor product was 2′,3′-O-isopropylidene-3,5′-cycloinosine (**9**). 5′-Azido-5′-deoxyinosine (**3**) could be readily obtained by acidic cleavage of the isopropylidene group of **2**. Catalytic hydrogenation of **3** with Raney nickel resulted in essentially complete conversion to 5′-amino-5′-deoxyinosine (**4**) which, as expected for an alkylamine, reacted with ninhydrin and migrated as a cation upon paper electrophoresis at neutral pH. The nucleoside **4** was conveniently obtained also by hydrogenation of **2** followed by acidic treatment of the resulting 5′-amino-2′,3′-O-isopropylidene-5′-deoxyinosine which was not isolated. This latter nucleoside could not be obtained by direct amination of either **1** or **5**.

Conversion of the tosyl derivative **1** to 5′-iodo-5′-deoxy-2′,3′-O-isopropylideneinosine (**5**) with NaI in acetone at 100° was reported by Levine and Tipson[9] but identification of the product was inadequate. Holmes and Robins[10] detected the cyclonucleoside **9** among products formed under the conditions of Levine and Tipson and did not report isolation of the 5′-iodo-substituted nucleoside **5**. Treatment of **1** with NaI in refluxing acetone furnished the nucleoside **5** in high yield. Removal of the isopropylidene group of **5** occurred smoothly at pH 2 to give 5′-iodo-5′-deoxyinosine (**6**). The iodo derivative could be converted to the corresponding 5′-thiocyanato derivative with sodium thiocyanate and to the 5′-mercapto derivative (**7**) with NaSH. Acidic treatment of **7** furnished 5′-mercapto-5′-deoxyinosine (**8**) in small yield, and paper chromatography indicated that this was due to extensive concomitant fission of the glycosidic bond. The blocked nucleoside **7** was totally converted to hypoxanthine by acidic conditions which had no effect on the glycosidic bond of 2′,3′-O-isopropylideneinosine (**10**).

Holmes and Robins[10] have shown that the tosyl derivative **1** is converted to 2′,3′-O-isopropylidene-3,5′-cycloinosine (**9**) in refluxing dioxane. In the present studies, this intramolecular reaction tended to accompany bimolecular displacement reactions at C-5′ of **1** and **5**, as noted above for the conversion of **1** to **2**. Attempts to prepare 5′-cyano-5′-deoxy-2′,3′-O-isopropylideneinosine by reaction of **1** or **5** with NaCN were unsuccessful, as judged by ir spectroscopy of the reaction products, and in some instances yielded principally the cyclonucleoside **9**. That formation of **9** is promoted by basic conditions was confirmed by the finding that **5** is converted to **9** at room temperature by NH_4OH; the ease of cyclonucleoside formation is presumably related to electron availability at N-3 in the monoanion of **5** as illustrated by structure **12**.

(1) (a) Presented in part at the 155th National Meeting of the American Chemical Society, Division of Medicinal Chemistry, San Francisco, Calif., April 1968. This work was supported by the National Cancer Institute of Canada and the Medical Research Council (Canada) (Grant MA-1591). (b) Author to whom enquiries should be addressed at The Institute for Cancer Research, Philadelphia, Pa. 19111.

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