complete destruction of the metal–porphyrin complexes, the durability of the porphyrin components of the crude oils and asphalts was unexpected. The data (footnote *b*) indicate that simple shielding by the petroleum is an insignificant factor as expected from the energy range of gamma radiation from spent fission products.[1] Crude petroleum may afford some protection against radiation-induced oxidation. However, the data (footnote *c*) indicate that the metal–porphyrin complexes are extensively decomposed even under anaerobic conditions. The metal–porphyrin complexes were separated from the petroleum by mild physical methods.[2] The possibility remained that the complexes in the petroleum were chemically different from those isolated. However, the enriched petroleum samples suffered essentially the same degree of decomposition as did the natural samples.

These data are interpreted as indicating that crude petroleum acts as a "protective" solvent for the metal–porphyrin complexes. Such action has been the subject of considerable research[3] but is only incompletely understood.[4] However, it appears that petroleum may be a natural protective agent against gamma irradiation by either of the commonly accepted mechanisms[5-6] in which it scavenges free radicals or provides an effective medium for internal energy transfer. The protective action of petroleum may have considerable practical importance in the development of nuclear engineering processes.

The author gratefully acknowledges the assistance of Mr. J. W. Moore in making the porphyrin analyses listed above.

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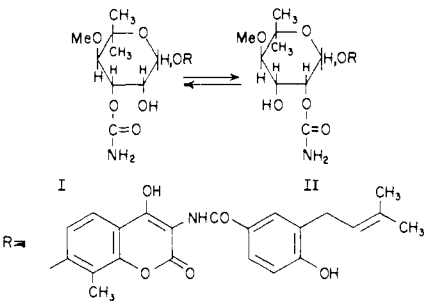
RECEIVED AUGUST 23, 1957

NOVOBIOCIN.[1] V. CARBAMYL MIGRATION AND ISONOVOBIOCIN

Sir:

The studies reported here show that novobiocin (I) isomerizes to isonovobiocin (II) in dilute alkali and establish the structure of II as 4-hydroxy-3-[4-hydroxy-3-(3-methyl-2-butenyl)-benzamido]-8-methyl-2-oxo-2*H*-1-benzopyran-7-yl 2-*O*-carbamyl-4-*O*-methyl-5,5-dimethyl-L-lyxoside.

When a solution of novobiocin was held at *p*H 10 for 2 hours at 25°, statistically significant bioassays indicated a loss of 30–35% of its antibiotic activity. The amorphous acid which was isolated was indistinguishable from *amorphous* I by ultraviolet measurements, elemental analyses and specific rotation. The amorphous nature of the material impaired infrared comparison, but no interpretable differences were detected. This ap-



parently homogeneous material was partially resolved, however, by countercurrent distribution into 2 main components after 2000 transfers using a solvent system comprising water, acetone, methyl ethyl ketone, and Skellysolve B (3:9:2:6 by volume). Analysis of the distribution data[2] showed the mixture contained *ca.* 67% of I and *ca.* 33% of a new material. This inactive component, very similar in physical properties to I, was named isonovobiocin.

When pure II was subjected to the original isomerization conditions, it was converted back to I in 55–60% yields. An equilibrium was thus indicated. Since no spectral changes were detected, it seemed most likely that the sugar moiety was involved.

The material used for the structure determination of II was obtained as follows: With the knowledge gained from the initial countercurrent distribution experiment a fractional crystallization procedure was developed to remove most of I from the equilibrium mixture. The remaining mixture was separated by countercurrent distribution (2000 transfers) and found to contain about 65% II, 22% I, and 13% sodium chloride and unidentified materials.

The purified isonovobiocin was heated under reflux in methanol containing a slight excess of one equivalent of acetyl chloride,[3] cleaving the molecule selectively to crystalline novobiocic acid (IV) in 98% yield and the methyl glycoside (III). The novobiocic acid was identical with that obtained from I by infrared, ultraviolet and melting point comparisons.

Compound III, crystallized from acetone and Skellysolve B, was found to be isomeric with methyl 3-*O*-carbamyl novioside[1a,3] (calcd. for $C_{10}H_{19}NO_8$: C, 48.19; H, 7.68; N, 5.62. Found: C, 48.46; H, 7.59; N, 5.54). It was different on the basis of infrared absorption. Barium hydroxide at 25° converted III to methyl novioside (V)[1c,3] in 96% yield, showing that III differed from methyl 3-*O*-carbamylnovioside only in the position of the carbamyl group. Acid hydrolysis of III yielded an

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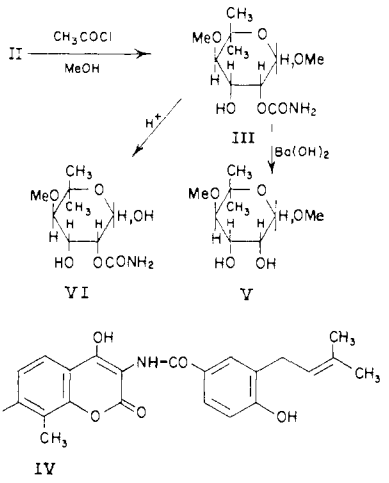
aldose which failed to react with periodate. These findings along with published data[1] permit the assignment of the structure of methyl 2-*O*-carbamyl-4-*O*-methyl-5,5-dimethyl-L-lyxoside to III and show that II is the 2-carbamyl analog of I.



It appears, therefore, that the antibiotic activity of novobiocin is highly dependent on the presence and location of the carbamate group.

We gratefully acknowledge the contributions of Drs. W. G. Jackson, W. T. Sokolski, J. L. Johnson, Mrs. G. S. Fonken, and Mr. W. A. Struck.

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RECEIVED AUGUST 8, 1957

AN INTERPRETATION OF THE PROTON MAGNETIC RESONANCE SPECTRUM OF RIBONUCLEASE

Sir:

Saunders, Wishnia and Kirkwood[1] recently have reported the first proton magnetic spectrum due to a protein, pancreatic ribonuclease, in D_2O. The spectrum consists of four broad peaks, falling roughly into the range between the aromatic and the aliphatic peaks of toluene. The authors have identified the first peak tentatively, corresponding to lower field strength, as due to the aromatic hydrogens and the fourth peak, at highest field strength as due to "aliphatic carbon atoms attached only to other aliphatic carbons," stating that a complete interpretation is not possible at present. However, our studies on the proton magnetic resonance spectra of amino acids and peptides[2,3] together with the amino acid composition of ribonuclease determined by Hirs, Stein and Moore,[4] permit the prediction of a complete n.m.r. spectrum for the protein, which is in excellent agreement with experiment.

There is little reason to doubt that peak I is due essentially to the 18 phenylalanine and 36 tyrosine hydrogens, as suggested.[1] In aqueous solutions these shifted -20 to -30 c.p.s. relative to the aromatic proton of toluene as 0. It probably also includes the 8 CH hydrogens on the imidazole of histidine, found at somewhat lower fields. The total number of non-exchangeable hydrogens in the protein being 697,[4] this will account for 8.9% of the total area under the absorption curve, in agreement with the $9 \pm 1\%$ measured. While in water the guanidino group of arginine gives rise to an additional peak in the same region, this disappears after equilibration with D_2O, so that it would not likely contribute to peak I of ribonuclease in this instance.

A second peak, due to the 123 α-CH protons, of all amino acids the 30 β-protons of serine and 10 β-protons of threonine should be observed at $+90$ to $+110$ c.p.s. relative to toluene if no shifts due to peptide bond formation were to be expected. In addition, the 6 glycine protons and 8 protons of the CH_2 group in arginine are observable at slightly higher fields of $+117$ to $+120$ c.p.s. in a region intermediate between the second and third peaks. Together with the protons of the second peak they would account for 25.4% of the total area, as compared to the experimental estimate of $26 \pm 2\%$. As peptide bond formation causes a displacement of α-CH peaks 15–20 cycles toward lower fields, the maximum of the second peak of ribonuclease, should appear at about $+80$ to $+90$ c.p.s. where it is actually found.

A third peak with a maximum between $+135$ to $+145$ c.p.s. is accounted for by CH_2 groups as follows: 16 from cysteine–cystine, 8 from methionine, 32 from aspartic acid, 20 from lysine (ϵ -CH_2), 10 from proline, 8 from histidine, 6 from phenylalanine and 12 from tyrosine. This amounts to 15.2% of the total area, the experimental estimate for the peak being $18 \pm 3\%$. The 64 CH_2 groups of glutamic acid fall at $+156$ c.p.s., accounting for an additional 9.2% of the area, which could be attributed either to the third or the fourth peak.

It is probable that peak IV is actually a fusion of two broad peaks, one with a maximum at $+180$ to $+190$ c.p.s., the other at $+210$ to $+220$. The first of these is due to aliphatic CH groups of leucine (2 hydrogens) isoleucine (3) and valine (9), CH_2 groups of leucine (4 hydrogens), isoleucine (6), proline (20), lysine (60) and arginine (16) and the CH_3 group of methionine (12 hydrogens), accounting for 19.1% of the area. The second, due to CH_3 groups of alanine (36 hydrogens), valine (54), leucine (12), isoleucine (18), and threonine (30) would respresent 21.6%. The combined area under peak IV, including glutamic acid is 49.9%, closely in agreement with the experimental value of $47 \pm 3\%$. It would be of interest if it were possible to sufficiently average the field seen by the aliphatic protons—perhaps at higher temperature—to actually resolve the fourth peak. It might also be noted, that due to peptide bond formation small and somewhat variable shifts toward lower fields can be expected for hydrogens contributing to peaks III and IV making an exact prediction of their maximum difficult. However, even the foregoing illustrates that NMR spectra

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