Mild basic hydrolysis of cyclobuxine triacetate led to the N,N′-diacetate (Ib), $C_{29}H_{46}O_3N_2 \cdot 2H_2O$, m.p. 283–285° dec., $[\alpha]^{24}D$ +10°, λ_{max} 2.95, 6.15 (very strong), 11.07 μ. Strong basic hydrolysis led in quantitative yield to an N-monoacetate (Ic), $C_{27}H_{44}O_2N_2$, m.p. 187–192°, strongly basic to phenol red, homogeneous upon chromatography, infrared 6.20 μ (strong). The remaining N-acetyl group could be removed only under vigorous acid conditions. The monoacetate, which could be reconverted to the N,N′-diacetate with acetyl chloride, gave upon oxidation a ketone which rapidly lost methylamine in base to give a material possessing the typical spectral characteristics of the cyclopentenone mixture encountered in the earlier structural investigations. Thus, the 20-N-acetyl group had been hydrolyzed, presumably assisted by a *cis*-interaction with the 16α-hydroxyl group.[14,15] In accord with this view, 16-dehydrocyclobuxine N,N′-diacetate (m.p. 222–225°, $[\alpha]^{22}D$ −39°) was recovered (60%) after treatment under the strong basic conditions. The foregoing facts support assignment of α-configuration to the 20-methylamino group, in good accord with biogenetic precedent.[16,17,18]

(14) *Cf.* S. M. Kupchan, S. P. Eriksen and M. Friedman, *J. Am. Chem. Soc.*, **84**, 4159 (1962).

(15) Facilitation of hydrolysis in a steroid 16α,20α-diacetate has been observed and attributed to the interference between the 18- and 21-methyl groups, forcing the 16α- and 20α-substituents to adopt parallel conformations. Thus, mild hydrolysis of 3β,16α,20α-pregnanetriol triacetate gave the 16,20-diacetate and the free triol. In contrast, mild hydrolysis of 3β,16α,20β-pregnanetriol triacetate gave a good yield of 20-monoacetate (H. Hirshmann and F. B. Hirshmann, *J. Biol. Chem.*, **184**, 259 (1950)).

(16) All known 20-amino steroids possess the α-configuration at that center: see R. Goutarel, *Tetrahedron*, **14**, 126 (1961), and O. Jeger and V. Prelog, in "The Alkaloids," ed. R. H. F. Manske, Vol. VII, Academic Press, New York, N. Y., 1960, pp. 319–342.

(17) Satisfactory analyses have been obtained for all compounds with cited empirical formulas. We thank Mr. Joseph Alicino, Metuchen, New Jersey, for the analyses.

(18) This investigation was supported in part by research grants from the National Institutes of Health (H-2952 and CY-4500).

(19) Cooperative National Science Foundation Predoctoral Fellow in Chemistry, 1960–1962.

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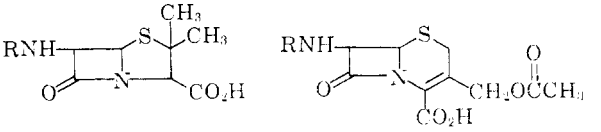
A NEW NATURAL PENICILLIN FROM *PENICILLIUM CHRYSOGENUM*

Sir:

Penicillium chrysogenum, when grown on suitable media, is known to produce a variety of penicillins.[1] Those formed are dependent upon the availability of suitable carboxylic acids as precursors and differ only in the nature of the N-acyl group attached to 6-aminopenicillanic acid (I, 6-APA). Such versatility may be related to the capacity of the organism to produce a relatively non-specific N-transferase.[2,3] With growth on a simple medium in the absence of added precursor acids, 6-APA is formed.[4] We wish to report the isolation of a new penicillin which may have significance in the biogenesis of this class of antibiotics.

When *Penicillium chrysogenum* was grown on a simple medium,[4] small amounts of solvent extractable penicillins were produced, along with 6-APA and another β-lactam type compound of low antibacterial activity. Isolation of the new compound (II) was achieved by adsorption on activated carbon (Norit SG) and elution with aqueous acetone, then chromatography on potato starch with aqueous *tert*-butyl alcohol. Peak fractions yielded a chromatographically homogeneous product which was indistinguishable from penicillin N (III)[5] in our chromatographic systems. It responded to the penicillinase-hydroxylamine assay, giving a color equivalent to *ca.* 1,200 μ/mg.[6] Antibacterial activity was low *vs. S. aureus* (209P) (compared to benzylpenicillin) and paralleled the activity of penicillin N.[7]

II was unstable in acid, being destroyed rapidly at pH 2. Destruction by penicillinase was rapid also, the rate of acid inactivation and penicillinase destruction being comparable to that observed for penicillin N. When paper chromatograms of II were sprayed with ninhydrin, a single major spot developed which coincided with the area of antibiotic activity. Treatment of II with mercuric chloride according to the procedure described by Abraham[8] for isolation of penillamine gave a mercaptide which was decarboxylated readily and formed a picrate. Hydrolysis with N hydrochloric acid, and preparative paper chromatography yielded L-α-aminoadipic acid, deduced from the facts that paper chromatographic behavior, infrared absorption curve, and X-ray diffraction pattern were identical with those of an authentic specimen of D-α-aminoadipic acid,[9] but the optical rotatory dispersion curve was equal in magnitude but opposite in sign to that given by the authentic sample.



I, R = H
II, R = L-$HO_2CCH(NH_2)(CH_2)_3CO$–
III, R = D-$HO_2CCH(NH_2)(CH_2)_3CO$–
IV, R = D-$HO_2CCH(NH_2)(CH_2)_3CO$–

(1) "Chemistry of Penicillin," Princeton University Press, 1948, Chapter 19. See also Q. F. Soper, C. W. Whitehead, O. K. Behrens, J. J. Corse and R. G. Jones, *J. Am. Chem. Soc.*, **70**, 2849 (1948), and preceding articles.

(2) W. H. Peterson and N. E. Wideburg, Proc. IVth International Congress of Biochemistry, 1958, p. 136.

(3) H. R. V. Arnstein and D. Morris, *Biochem. J.*, **76**, 357 (1960).

(4) K. Kato, *J. Antibiotics (Japan)*, *Sec. A*, **6**, 130 (1953). The medium used in our work was made up of 2% brown sugar, 0.6% $NaNO_3$, 0.05% $MgSO_4 \cdot 7H_2O$, 2.5% $CaCl_2$, 0.15% KH_2PO_4, and tap water. Incubation was at 25° on rotary shakers for eight days. See also ref. 12.

(5) Previously known as synnematin B and cephalosporin N, the name penicillin N was proposed for III by Demain and Newkirk and Trown, *et al.*, to eliminate this duality. See A. L. Demain and J. F. Newkirk, *Applied Microbiol.*, **10**, 321 (1962), and P. W. Trown, E. P. Abraham, G. G. F. Newton, C. W. Hale and G. A. Miller, *Biochem. J.*, **84**, 157 (1962).

(6) Benzylpenicillin which is used as a standard, has an arbitrarily assigned color potency of 1600 μ/mg. See J. H. Ford, *Ind. Eng. Chem., Anal. Ed.*, **19**, 1004 (1947).

(7) E. P. Abraham and G. G. F. Newton, *Biochem. J.*, **58**, 94 (1954).

(8) E. P. Abraham and G. G. F. Newton, *ibid.*, **58**, 109 (1954).

(9) We wish to thank Dr. Milton Winitz for supplying the D-α-aminoadipic acid and Max M. Marsh for determining the rotatory dispersion.

The chemical properties described indicate structure II for the compound, differing from penicillin N only in configuration at C_5'. We propose the name isopenicillin N for this antibiotic. Penicillin N has been described as a product of fermentation by *Cephalosporium* species, at least one member of which also is capable of producing cephalosporin C (IV)[10] concurrently with penicillin N. Penicillin N has not been reported as a product of *Penicillium* fermentation. Although both the *Penicillium* and *Cephalosporium* species are capable of synthesizing the 6-APA fragment (as evidenced by penicillin N from *Cephalosporium*) an antibiotic common to both species has not been described. The discovery of isopenicillin N suggests possible convergence of biosynthetic pathways; however, one differentiating factor may be stereochemistry of the aminoadipyl side chain. Arnstein and Morris[3] have found α-aminoadipic acid in extracts of *Penicillium* mycelium, the amino acid apparently possessing the L configuration. They also have reported isolation of δ-(α-aminoadipyl)-cysteinyl-valine but did not determine configuration of the aminoadipyl component. A biosynthetic scheme was proposed by them, based on isolation of the tripeptide and earlier work on biosynthesis of penicillin.[11] The presence of isopenicillin N in *Penicillium* fermentations is consonant with results reported in a later article by Wolff and Arnstein[12] and confirms their speculations concerning the presence of such a molecule.

(10) G. G. F. Newton and E. P. Abraham, *Biochem. J.*, **62**, 651 (1956).

(11) For leading references, see (3) and (12).

(12) E. C. Wolff and H. R. V. Arnstein, *Biochem. J.*, **76**, 375 (1960).

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1,8-BISDEHYDRO[14]ANNULENE, AN UNUSUAL AROMATIC COMPOUND[1]

Sir:

It has been reported that the oxidative coupling of *trans,trans*-4,10-tetradecadiene-1,7,13-triyne with cupric acetate in pyridine and subsequent treatment with potassium *t*-butoxide in *t*-butyl alcohol yielded two isomers of monodehydro[14]annulene (cyclotetradecahexaen-yne).[2] The isomer obtained in larger amount (which on partial hydrogenation had led to [14]annulene[2,3]) by nuclear magnetic resonance (n.m.r.) spectroscopy was subsequently shown to be a monodehydro[14]annulene containing 4 *cis* and 2 *trans* double bonds (*e.g.*, I).[4] It now has been found that the minor product is not an isomer of I, but in fact is 1,8-bisdehydro[14]annulene(II),[5] an unusual aromatic compound which can only be represented by cumulene containing Kekulé resonance forms.

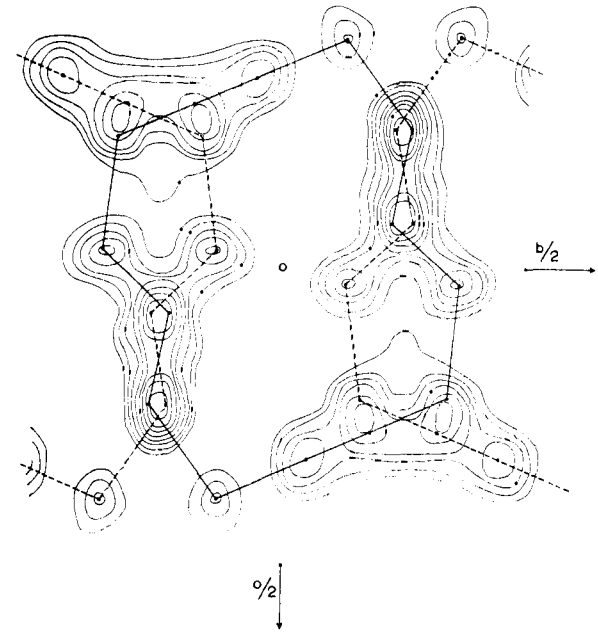
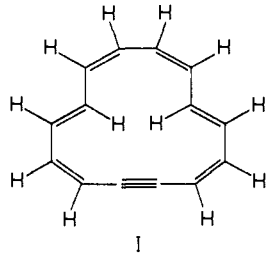
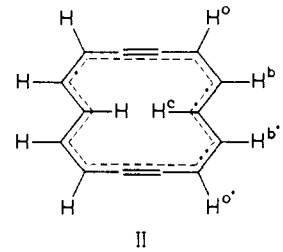


Fig. 1.—1,8-Bisdehydro[14]annulene (II): the electron density synthesis $\rho(XYO)$. The broken lines refer to molecules related by symmetry to the reference molecule.



I



II

The n.m.r. spectrum of 1,8-bisdehydro[14]annulene (II) in deuteriochloroform solution (determined with a Varian V 4300 spectrometer operating at 60 Mc/sec.) consists of three multiplets at 15.54, 1.57 and 0.45τ, with relative intensities of 2:4:4. Thus, the substance possesses 10 rather than 12 protons. The presence of two groups of equivalent protons suggests that the molecule is symmetrical, and this is supported by the fine structure of the multiplets, which also shows two blocks of five contiguous C–H groups each to be present. The band at 15.54 τ is assigned to H^o (see formula II), since these protons should experience strong shielding by the secondary magnetic field arising from the ring current. This band is a symmetrical triplet, with J = 13.3 c./s. This coupling constant also appears in the band at 0.45 τ, which is a double doublet, and is assigned to H^b and $H^{b'}$. The second splitting (J = 8.0 c./s.) of the 0.45 τ band is equal to that observed for the band at 1.57 τ, which is a doublet and must arise from H^a and $H^{a'}$. The relative values of J_{ab} and of J_{bc} are indicative of *cis*- and *trans*-interactions, respectively,

(1) This is part XXIX in the Weizmann Institute series "Unsaturated Macrocyclic Compounds." For part XXVIII, see F. Sondheimer and D. A. Ben-Efraim, *J. Am. Chem. Soc.*, in press.

(2) F. Sondheimer and Y. Gaoni, *ibid.*, **82**, 5765 (1960).

(3) J. Bregman, *Nature*, **194**, 679 (1962).

(4) L. M. Jackman, F. Sondheimer, Y. Amiel, D. A. Ben-Efraim, Y. Gaoni, R. Wolovsky and A. A. Bothner-By, *J. Am. Chem. Soc.*, in press.

(5) Although the original elemental analysis of II[3] had supported a monodehydro[14]annulene structure, another analysis (found: C, 93.94; H, 6.02) was more in accord with a $C_{14}H_{10}$ formula (calcd.: C, 94.34; H, 5.66) than a $C_{14}H_{12}$ formula (calcd.: C, 93.29; H, 6.71).