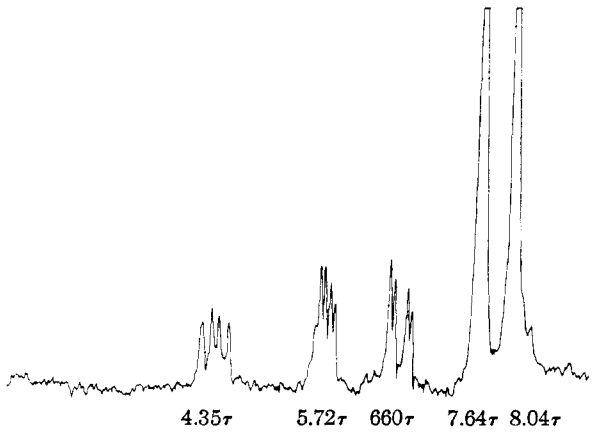
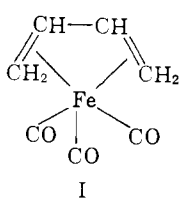


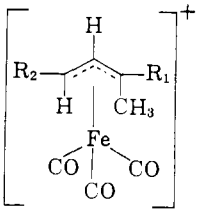
Fig. 1.—N.m.r. spectrum of 1,1-dimethyl-π-allyl-iron tricarbonyl fluoroborate.

fluorocyclohexenyl-iron tricarbonyl anion.[3] In contrast to these latter complexes the effective atomic number of krypton is not attained by iron in the present complexes.

The present series of salts has been obtained by reaction of acyclic diene-iron tricarbonyl complexes with strong acids. Contrary to a recent report,[4] such treatment results in protonation of a double bond already involved in coördination to the metal, rather than protonation on the metal atom. Thus reaction of butadiene-iron tricarbonyl (I) with HBF_4, $HClO_4$, or $HSbCl_6$ in nitromethane gives rise to the corresponding salts of the 1-methyl-π-allyl-iron tricarbonyl cation (II, $R_1 = R_2 = H$). (Analysis of the fluoroborate salt, calcd. for $C_7H_6FeO_3 \cdot HBF_4$: C, 29.83; H, 2.51; B, 3.84. Found: C, 29.66; H, 2.67; B, 3.75.) In a similar way the



I

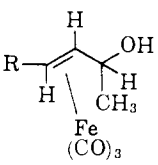


II

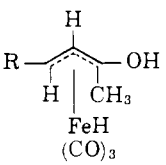
fluoroborate salts of the 1,1-dimethyl-π-allyl-Fe(CO)$_3$ cation (II, $R_1 = CH_3$, $R_2 = H$), the 1,3-dimethyl-π-allyl-Fe(CO)$_3$ cation (II, $R_1 = H$, $R_2 = CH_3$), and the 1-methyl-3-phenyl-π-allyl-Fe(CO)$_3$ cation (II, $R_1 = H$, R_2 = phenyl) have been made from isoprene-iron tricarbonyl, *trans*-piperylene-iron tricarbonyl and 1-phenylbutadiene-iron tricarbonyl, respectively.[5]

All of these salts are pale yellow, crystalline, diamagnetic solids, very soluble in nitromethane and liquid SO_2 but insoluble in non-polar solvents. They each exhibit strong carbonyl absorption frequencies in the infrared at about 2085 and 2150 cm.$^{-1}$, and none at lower frequencies commonly associated with a bridging carbonyl group.[6] Evidence for the structure of the cations is seen in the n.m.r. spectra of the salts taken in SO_2. For example the spectrum of the complex derived from isoprene-iron tricarbonyl is shown in Fig. 1; the two singlet methyl peaks at 764 and 8.04 τ are clearly in accord with structure II ($R_1 = CH_3$, $R_2 = H$) and the other protons are readily assigned from the observed splitting pattern. The methyl group of the complex derived from butadiene-iron tricarbonyl gives rise to a doublet centered at 8.17 τ while those of protonated piperylene-iron tricarbonyl show as two doublets centered at 7.86 and 8.35 τ.[7] This latter result indicates that geometrical inversion does not accompany protonation. The cryoscopic measurements of butadiene-iron tricarbonyl in H_2SO_4 indicate an i-factor of 2.16 ± 0.16. The n.m.r. spectrum of the solution in H_2SO_4 is similar to that of the salt in liquid SO_2 and shows no proton resonance at abnormally high fields indicative of a proton attached to a metal.[4]

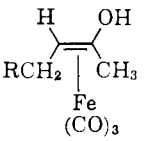
Of further interest are the products obtained after reaction of the salts with water. For example, such treatment of the salt derived from butadiene-Fe(CO)$_3$ gives rise to appreciable quantities of 2-butanone while that derived from *trans*-piperylene-Fe(CO)$_3$ gives 2-pentanone. These products probably result from attack of water to give a substituted allyl alcohol complex (III) and subsequent isomerization, *via* π-allyl-hydroiron tricarbonyl complexes (IV), to enol-Fe(CO)$_3$ complexes (V). The latter are expected to be unstable and would decompose to give the observed products.



III



IV



V

In agreement with this, the salt derived from isoprene-Fe(CO)$_3$ gives mainly dimethylvinylcarbinol; in this instance isomerization cannot occur because of the absence of a hydrogen on the carbon atom bearing the hydroxyl group. Also consistent with this mechanism is the isomerization of allyl alcohol to propionaldehyde observed when the alcohol is heated with Fe(CO)$_5$.

We thank the National Science Foundation and the Robert A. Welch Foundation for financial support.

(3) H. H. Hoehn, L. Pratt, K. F. Watterson and G. Wilkinson, *J. Chem. Soc.*, 2738 (1961).

(4) A. Davison, W. McFarlane, L. Pratt and G. Wilkinson, *Chem. and Ind.*, 553 (1961).

(5) Satisfactory analyses were obtained for these other salts.

(6) Bands observed at 1510–1525 cm.$^{-1}$ may be associated with the π-allyl group; *cf.* reference (1).

(7) N.m.r. spectra were taken at 60 Mc. Peak intensities of olefin and methyl protons, in all cases, were found in the expected ratios.

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RECEIVED SEPTEMBER 4, 1962

THE CONFIGURATION OF CYCLOBUXINE AND ITS INTERRELATION WITH CYCLOEUCALENOL

Sir:

In our previous communication,[1] we proposed structure Ia, exclusive of stereochemistry, for cyclobuxine, the major alkaloid of the acetone-insoluble

(1) K. S. Brown, Jr., and S. M. Kupchan, *J. Am. Chem. Soc.*, **84**, 4590 (1962).

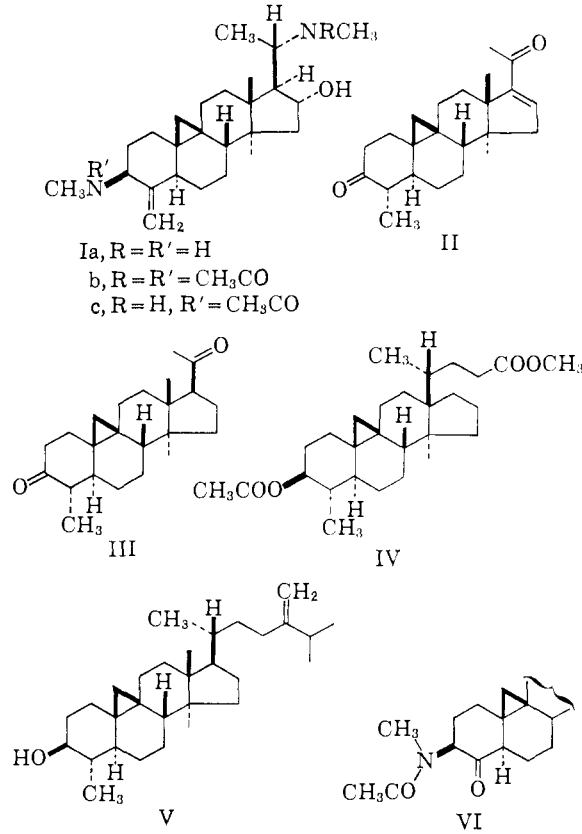
fraction of the strong bases from *Buxus sempervirens* L. We herewith present evidence for configuration Ia for cyclobuxine, which may be assigned the systematic name 3β,20α-di-(methylamino)-4α,14α-dimethyl-9β,19-cyclo-$\Delta^{4,4a}$-5α-pregnen-16α-ol.

Ruschig degradation[2] of dihydrocyclobuxine[1] led smoothly through a crystalline dichloramine ($C_{25}H_{42}ON_2Cl_2 \cdot 0.5H_2O$, dec. > 150°) to the one-enone (II) (λ_{max} 5.87, 6.01, 6.28 μ,[3] λ_{max}^{EtOH} 243 mμ (ϵ 7,700)), which upon selective hydrogenation gave *4α,14α-dimethyl-9β,19-cyclo-5α-pregnane-3,20-dione* (III), $C_{23}H_{34}O_2$, m.p. 182–184°, $[\alpha]^{23}D$ +114°, λ_{max} 5.87 (very strong). This was identical (m.p., mixed m.p., infrared spectrum, and chromatographic behavior (paper, partition, and adsorption)) to the compound obtained from Meystre–Miescher degradation[4] (with the final oxidation performed on the 3-alcohol) of the tetranor acid methyl ester acetate (IV), obtained by essentially the published route from an authentic sample of cycloeucalenol (V).[5] As cycloeucalenol has been related to cycloartenol[5] and lanosterol,[6] the foregoing not only constitutes strong support for the proposed structure for cyclobuxine, but also establishes the absolute configuration at six of its ten asymmetric centers, namely, 5α, 8β, 9β, 10β, 13β, and 14α.

Evidence is advanced for assignment of configuration at each of the four remaining centers. The ozonolysis product of cyclobuxine triacetate (VI), $C_{30}H_{46}O_5N_2$, dec. 225°, $[\alpha]^{23}D$ − 11°, had an o.r.d. curve demonstrating a weak negative Cotton effect (M_{316} = −2750°) similar to that of cholestane-4-one ($M_{307.5}$ = −3000°).[7] Furthermore, the 3-monomethiodide of dimethyldihydrocyclobuxine ($C_{28}H_{51}ON_2I$) melted at 245–255° without evolution of trimethylamine, and failed to undergo Hofmann degradation, giving under vigorous conditions a 60% recovery of dimethyldihydrocyclobuxine and no detectable olefin. The o.r.d. curve and the behavior upon Hofmann degradation[8] favor assignment of equatorial conformation (hence β-configuration) to the 3-methylamino group. The 5α-assignment was also supported by the above o.r.d., and by the difference in molecular rotation, −496°, between the Hofmann degradation product of cyclobuxine[1] and its tetrahydro derivative.[1,9]

The α-orientation of the 16-hydroxyl was suggested by the results upon acetylation of a variety of nitrogen-protected derivatives, which led to an average change in molecular rotation (for six pairs) of about −140°.[10] Support came in the sodium borohydride reduction of 16-dehydro-N,N′-dibenzoyldihydrocyclobuxine[1] (obtained by oxidation of N,N′-dibenzoyldihydrocyclobuxine, m.p. 273–275° dec., $[\alpha]^{28}D$ −27°) to 16-epi-N,N′-dibenzoyldihydrocyclobuxine, m.p. 260–261° dec., $[\alpha]^{26}D$ −31°, characterized by oxidation back to the 16-dehydro compound. Acetylation of the 16-epi-alcohol was accompanied by a strong positive molecular rotation increment, in accord with a 16β-configuration.[11]

The o.r.d. curves of various 16-ketones showed the strong negative Cotton effect, with the trough far out and a shoulder about five mμ below it, typical of 16-keto-17α-H steroids.[12,13] In contrast, a 16-keto-17β-H derivative prepared during the course of this work demonstrated a moderately strong positive Cotton effect (M_{334} = +4500°). The n.m.r. signal of the 16β-hydrogen in cyclobuxine is split by one nearly opposing proton (J 9.5 c./s.); models indicate that this could only occupy the 17α-position.



(2) H. Ruschig, W. Fritsch, J. Schmidt-Thomé and W. Haede, *Chem. Ber.*, **88**, 883 (1955); L. Lábler and F. Sorm, *Coll. Czech. Chem. Comm.*, **24**, 2975 (1960).

(3) All rotations and infrared spectra are in chloroform.

(4) Ch. Meystre, H. Frey, A. Wettstein and K. Miescher, *Helv. Chim. Acta*, **27**, 1815 (1944); C. S. Barnes, *Austr. J. Chem.*, **9**, 228 (1956).

(5) J. S. G. Cox, F. E. King and T. J. King, *J. Chem. Soc.*, 1384 (1956); 514 (1959). We thank Dr. T. J. King for a generous gift of cycloeucalenol for this degradation.

(6) H. R. Bentley, J. A. Henry, D. S. Irvine and F. S. Spring, *ibid.*, 3673 (1953); D. S. Irvine, J. A. Henry and F. S. Spring, *ibid.*, 1316 (1955).

(7) C. Djerassi, "Optical Rotatory Dispersion," McGraw-Hill, New York, N. Y., 1960, pp. 43, 50-51.

(8) *Cf.* R. D. Haworth, J. McKenna and R. G. Powell, *J. Chem. Soc.*, 1110 (1953).

(9) ΔM_D for the transformation cyclobuxine → dihydrocyclobuxine is −205°; thus, the increment for saturation of the 2,3-double bond is −291°. The listed values are, for 5α, −152°; for 5β, +24° (W. Klyne, in E. A. Braude and F. C. Nachod, "Determination of Organic Structures by Physical Methods," Academic Press, New York, N. Y., 1955, p. 111).

(10) Average, when there is a 17β-side chain, for 16α-OH to 16α-OAc, −240°; for 16β-OH to 16β-OAc, +65° (D. K. Fukushima and T. F. Gallagher, *J. Am. Chem. Soc.*, **78**, 196 (1951)). See also ref. 15.

(11) Stereospecific rear attack at C-16, to yield 16β-alcohols, has been noted previously for a variety of reducing agents, including lithium aluminum hydride; *cf.* S. Bernstein, M. Heller and S. M. Stolar, *ibid.*, **77**, 5327 (1955).

(12) *E.g.*, the 16-dehydro-N,N′-dibenzoyldihydrocyclobuxine mentioned above: trough M_{331} = −6300°, shoulder $M_{325-320}$ = −6000°.

(13) Reference 7, pp. 44–45, 57–58; C. Djerassi, R. Riniker and B. Riniker, *ibid.*, **78**, 6362 (1956).

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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RECEIVED OCTOBER 26, 1962

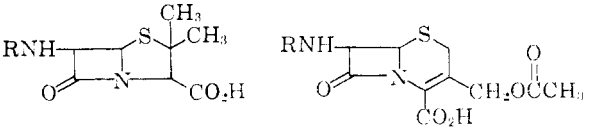
A NEW NATURAL PENICILLIN FROM *PENICILLIUM CHRYSOGENUM*

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.