Fungal Metabolites. The Structures of the Novel Sesquiterpenoids Illudin-S and -M

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From a study of the chemical and spectral properties of the fungal metabolites illudin-S and -M, and of several of their transformation products, unique structures (XV, XVI) have been assigned the two compounds. They are shown to be terpenoid in nature and a hypothesis is proposed for their elaboration from mevalonic acid.

The basidiomycete *Clitocybe illudens*, commonly known as the Jack-o'-lantern mushroom because of its bioluminescent property, occurs in large clusters in late summer and autumn particularly in the eastern United States. It has a beautiful saffron-yellow color and is toxic. There have been reports of severe illness caused by eating the mushroom.[1]

In the course of an investigation of antibacterial substances produced by higher fungi two crystalline compounds, designated illudin-S and -M, were isolated from culture liquids of *C. illudens*. They were toxic and possessed antibacterial activity.[2] An examination undertaken by the National Cancer Institute, National Institutes of Health, during the past few years has shown that the compounds also possess antitumor activity. Illudin-S and -M are sesquiterpenoids with novel structures, the evidence for which forms the subject of this paper.[3]

Illudin-S, $C_{15}H_{20}O_4$, and illudin-M, $C_{15}H_{20}O_3$, have nearly identical ultraviolet spectra, λ_{max} 233 and 319 mμ (ϵ 13,200 and 3600) and λ_{max} 228 and 318 mμ (ϵ 13,900 and 3600); these suggested the presence of cross-conjugated dienone.

The infrared spectra were consistent with this, showing ν_{max} 1706 (s), 1652 (w), 1610 (s) cm.$^{-1}$ and 1695 (s), 1661 (w), and 1595 (s) cm.$^{-1}$, respectively. The presence of three hydroxyl groups in illudin-S was indicated by formation of a diacetate which still showed hydroxyl absorption (ν_{max} 3550 cm.$^{-1}$). Illudin-M gave a monoacetate containing a free hydroxyl. All the oxygen atoms in the two compounds are thus accounted for.

Catalytic hydrogenation of illudin-S with palladized charcoal in methanol proceeded with rapid uptake of roughly 1.5 moles of hydrogen, followed by further slow absorption. The noncrystalline product obtained after 1.5 moles uptake exhibited phenolic absorption (λ_{max} 280 mμ) which changed very quickly on boiling the dilute alcoholic solution with a trace of acid. A steam-volatile crystalline compound was formed and the residue which precipitated was partly crystalline on cooling.

(1) See, for example, J. Walton Groves, "Edible and Poisonous Mushrooms of Canada," Research Branch, Canada Department of Agriculture, Ottawa, Ontario, 1962, p. 120.

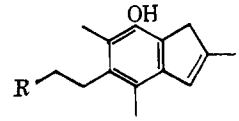
(2) M. Anchel, A. Hervey, and W. J. Robbins, *Proc. Natl. Acad. Sci. U. S.*, **36**, 300 (1950); **38**, 927 (1952).

(3) For a preliminary communication of this evidence, see T. C. McMorris and M. Anchel, *J. Am. Chem. Soc.*, **85**, 831 (1963).

The steam-volatile compound, $C_{14}H_{18}O$, could be assigned the structure I except for the orientation of substituents on the benzene ring. In I and in other indene and indan derivatives to be described, this orientation was deduced from the structures of illudin-S and -M. Thus I has λ_{max} 266, 300, and 311 mμ (ϵ 9700, 2800, and 2600); it is soluble in alkali and it formed a monoacetate (ν_{max} 1757 cm.$^{-1}$). The n.m.r. spectrum has signals for 18 protons. There are two aromatic methyls (τ 7.79 and 7.72) and an aromatic ethyl (τ 7.33 and 8.92, quartet and triplet, J = 7.5 c.p.s.). The broad singlet at 6.83 is assigned to the cyclic methylene and the quartet at 3.48 (J = 1.5 c.p.s.) to the olefinic proton. The olefinic methyl gives the broad singlet at τ 7.89 and the hydroxyl the very broad peak at 5.65.

The crystalline residue (II) from acid treatment of the hydrogenation product analyzed for $C_{15}H_{20}O_2$ and has virtually the same ultraviolet spectrum as I. The n.m.r. spectrum has a singlet at τ 6.83, indicating the presence of an aliphatic methoxyl, and there is no ethyl present. Since the parent illudin-S does not possess a methoxyl it seemed that the methoxyl in II might have come from the solvent. In agreement, the hydrogenation carried out in ethanol led to a corresponding product, $C_{16}H_{22}O_2$ (III), which gives signals at τ 6.48 and 8.78 (quartet and triplet, J = 7 c.p.s.), expected for an ethoxyl.

In the n.m.r. spectrum of II there is a multiplet in the region 6.3–7.25 which can be assigned to the methylene protons in the group $-O-CH_2-CH_2-Ar$. Likewise for III a multiplet occurs at 6.25–7.2 assignable to $-O-CH_2-CH_2-Ar$. Compounds II and III could therefore be represented as

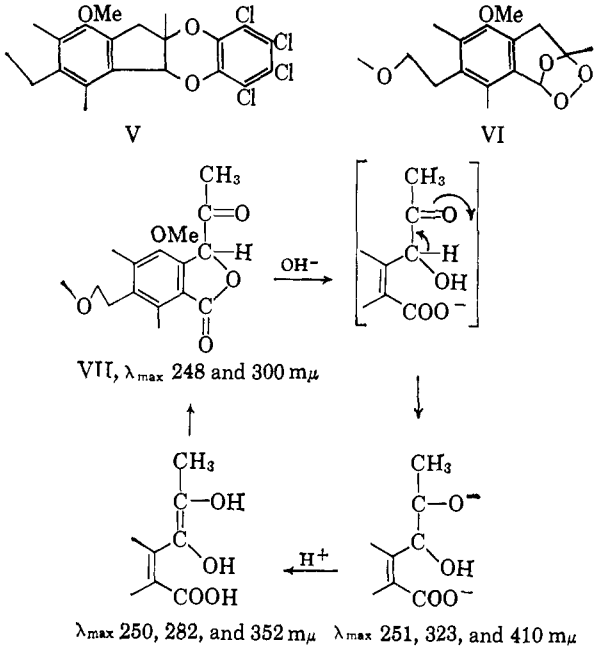


I, R=H
II, R=OMe
III, R=OEt
IV, R=Cl

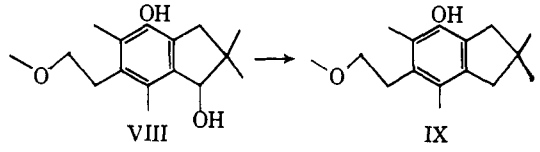
The indenols I, II, and III were also obtained in another way. Addition of dilute sulfuric acid to a refluxing solution of illudin-S in methanol, to which zinc dust had previously been added, caused rapid disappearance of the dienone chromophore and development of the indenol one. Dilution with water afforded a crystalline precipitate of II in good yield (~68%). With ethanol as solvent, III was obtained on similar treatment. When hydrobromic or hydriodic acid was used instead of sulfuric acid, the volatile indenol I was the main product. Hydrochloric acid gave a chlorinated indenol which is assigned structure IV.

Consistent with the indenol formulation for I–IV were a number of reactions. Catalytic hydrogenation gave the corresponding indanols with the expected spectral properties. The methyl ether of I readily formed an adduct (V) with tetrachloro-1,2-benzoquinone, which was characterized as a 1,4-dioxene by its infrared spectrum (ν_{max} 1425 cm.$^{-1}$).[4] On treatment with ozone, the methyl ether of II afforded a stable crystalline ozonide, $C_{16}H_{22}O_5$ (VI).[5] This gave with chromium trioxide in aqueous acetic acid a product, $C_{16}H_{20}O_5$ (VII), formulated as a phthalide on the basis of its spectral properties. It shows ν_{max} 1770 and 1730 cm.$^{-1}$, λ_{max} 248 and 300 mμ. Addition of a drop of alkali to the ethanolic solution gave a bright yellow solution having λ_{max} 251, 323, and 410 mμ. The color disappeared on acidification, and the solution then had λ_{max} 250, 282, and 352 mμ. When this solution was allowed to stand for about 2 hr., the spectrum changed back entirely to that of the starting compound. These properties are rationalized in Scheme I below. The n.m.r. spectrum is in accord with the assigned structure.

Scheme I



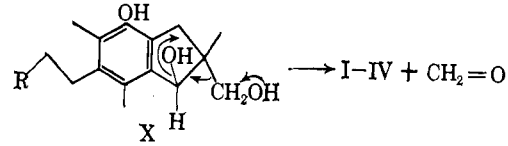
Illudin-M behaved in the same way as illudin-S on catalytic hydrogenation, but the product obtained after 1.5 moles uptake was partly crystalline and yielded a diol, $C_{16}H_{24}O_3$, whose spectral properties indicated the structure VIII. It contains a methoxyl group and it formed a diacetate (ν_{max} 1760 and 1733



cm.$^{-1}$) (refluxing an acetic acid solution of VIII gave the monoacetate, ν_{max} 1736 cm.$^{-1}$). On catalytic hydrogenation VIII gradually took up 1 mole of hydrogen giving a phenol $C_{16}H_{24}O_2$ (IX). This hydrogenolysis of the benzylic hydroxyl in VIII was accompanied by the appropriate changes in the n.m.r. spectrum. The *gem*-dimethyl now appears as a sharp singlet at 8.82 instead of the two singlets at τ 8.76 and 8.98 for VIII, and the two pairs of cyclic methylene protons in IX give singlets at τ 7.30 and 7.37.

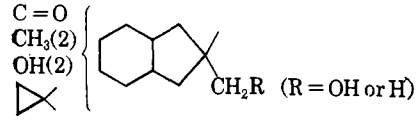
The origin of the group R–CH_2–CH_2– (R = H, OMe, OEt, or Cl) in the aromatic compounds derived from the illudins is evident from the n.m.r. spectra of the latter. They each show a multiplet at high field consistent with the presence of about four cyclopropyl protons. A spirocyclopropane ring on cleavage would give just such a fragment, R–CH_2–CH_2–.[6]

The spectra also show that illudin-S has three, and illudin-M four methyl groups. Illudin-S gives a singlet at τ 6.53 due to the methylene protons of a hydroxymethyl group. This assignment is confirmed by acetylation which causes a shift downfield to τ 5.94. (The signal is now an AB quartet due to restricted rotation of the –CH_2OAc.) Evidently the hydroxymethyl takes the place of one of the methyls of the *gem*-dimethyl in illudin-M so that primary hydrogenation products of illudin-S can be represented by X.

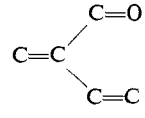


Cleavage of the 1,3-diol ("reverse Prins reaction") occurs on acid treatment giving an indenol and formaldehyde. (Repetition of the reactions leading to the indenols I–IV gave formaldehyde, isolated as its dimedone derivative, in fair yield.) This very facile elimination favors a structure with the *secondary* hydroxyl and hydroxymethyl *trans* to one another.[7] (A similar relationship would hold in illudin-S.)

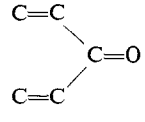
Evidence presented so far allows one to write the following partial structure for the illudins



Reduction of illudin-M with sodium borohydride afforded a crystalline triol, $C_{15}H_{22}O_3$ (XI), which contains a conjugated diene (λ_{max} 256 mμ (ϵ 22,500)).[8] It follows that the chromophore in the illudins has the arrangement



rather than



(4) L. M. Jackman in "Advances in Organic Chemistry; Methods and Results," Vol. 2, Interscience Publishers, Inc., New York, N. Y., 1960, p. 335.

(5) Unsaturated five-membered ring systems are known to give stable ozonides whereas bigger ring compounds yield largely polymeric ozonides and/or peroxides. See P. S. Bailey, *Chem. Rev.*, **58**, 929 (1958).

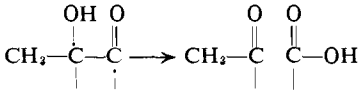
(6) *Cf.* spiro[2.5]octa-1,4-dien-3-one: R. Baird and S. Winstein, *J. Am. Chem Soc.*, **79**, 4238 (1957); **85**, 567 (1963).

(7) For studies on the mechanism of the cleavage of 1,3-diols see T. E. Maggio and J. English, Jr., *ibid.*, **83**, 968 (1961).

(8) This ultraviolet spectrum indicates that the cyclopropane in conjugation with the transoid diene in XI (see later) causes only a small bathochromic shift. *Cf.* 3,5-cyclo-$\Delta^{6,8(14),22}$-ergostatriene: L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p. 318.

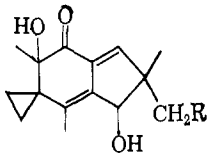
and one of the double bonds must therefore be in the five-membered ring (the carbonyl group is excluded from this ring; *vide infra*).

On treatment with sodium metaperiodate the illudins readily gave crystalline keto acids (XII, XIII). Thus XII, $C_{15}H_{20}O_5$, effervesced with sodium bicarbonate solution and has ν_{max} 1710 (carboxyl) and 1684 cm.$^{-1}$ (cyclopropyl ketone) indicating the conversion



Periodate oxidation of the triol XI afforded a crystalline keto aldehyde (XIV) whose n.m.r. spectrum clearly shows the A_2B_2 multiplet at about 8.65 and 9.2 due to the spirocyclopropane. The diol acetate obtained by borohydride reduction of illudin-M acetate also gave a keto aldehyde on periodate oxidation. In an earlier experiment, an attempt to convert the diol acetate back to illudin-M acetate by treatment with activated manganese dioxide in chloroform yielded instead the acetate of XIV. On similar treatment the triol XI furnished the keto aldehyde XIV. (Illudin-M was recovered unchanged under these conditions.)

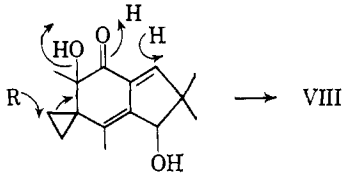
The presence of an α-ketol and a cross-conjugated dienone leads to the unique structures XV and XVI for illudin-S and -M.

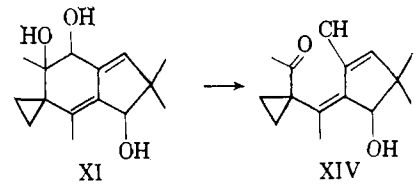


XV, R = OH
XVI, R = H

The n.m.r. spectra are fully consistent with XV and XVI. In that of XV, signals at τ 6.43 and 8.0 are due to three hydroxyl protons (as determined by exchange with D_2O). The olefinic proton appears at τ 3.55, the proton α to hydroxyl (secondary) at 5.3, the olefinic methyl at 8.32, and the methyl α to hydroxyl at 8.65. Similar assignments hold in the spectrum of XVI. The *gem*-dimethyl gives singlets at τ 8.81 and 8.92, the former probably due to that methyl which is *cis* to the secondary hydroxyl. Significantly, there is a corresponding singlet (at τ 8.82) in the spectrum of XV. This provides further indication that the hydroxymethyl is *trans* to the secondary hydroxyl in illudin-S.

The structures XV and XVI account for the remarkable reactivity of the illudins. In particular, the conversion to aromatic compounds can now be rationalized, and the orientation of substituents in the benzene ring of I–IX is thus derived. Compounds XI and XIV have the structures

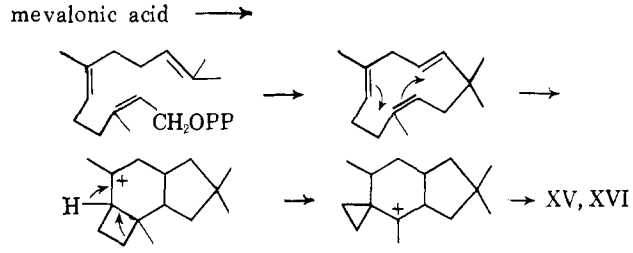




XI XIV

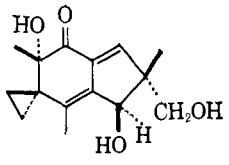
Inspection of XV and XVI suggests that the illudins are terpenoid in nature and a hypothetical scheme (Scheme II) for their biogenesis from farnesyl pyrophosphate, or its biochemical equivalent, may be formulated as

Scheme II



Scheme II raises the interesting possibility that humulene, the eleven-membered ring hydrocarbon whose stereochemistry is now settled,[9] may be an intermediate in the biosynthesis. Feeding experiments with *C. illudens* have resulted in the incorporation 2-C^{14}-mevalonic acid into illudin-S and -M, and further experiments are in hand to locate the active centers in the sesquiterpenoids.

A recent report from Japan[10] tells of an antitumor compound isolated from the poisonous mushroom *Lampteromyces japonicus* (Kawam) Sing.[11] which has been found to be identical with illudin-S. An X-ray analysis on the *p*-iodobenzoate of its acyloin rearrangement product, together with optical data, define the absolute stereochemistry as shown.



Experimental[12]

Illudin-S (XV). This compound crystallized readily from common organic solvents and from water. It formed needles from acetone, m.p. 124–125°,[2] and prisms from ethyl acetate, m.p. 137–138°. The higher melting material gave satisfactory analyses. *Anal.*

(9) A. T. McPhail, R. I. Reed, and G. A. Sim. *Chem. Ind.* (London), 976 (1964); J. A. Hartsuck and I. C. Paul, *ibid.*, 977 (1964).

(10) M. Tada, Y. Yamada, N. S. Bhacca, K. Nakanishi, and M. Ohashi, *Chem. Pharm. Bull.* (Tokyo), **12**, 853 (1964); K. Nakanishi, M. Tada, and Y. Yamada, *ibid.*, **12**, 856 (1964).

(11) This mushroom is bioluminescent and it belongs to the same family, *Tricholomataceae* (Singer), as *C. illudens*.

(12) Melting points were taken on a Köfler hot stage. Infrared spectra were determined with a Perkin-Elmer Model 21 spectrophotometer, in potassium bromide disks except where indicated. Ultraviolet spectra were measured in ethanol with a Cary Model 11 spectrophotometer. N.m.r. spectra were determined in deuteriochloroform on an A-60 spectrometer by Dr. D. P. Hollis, Varian Associates, Calif. Microanalyses were carried out by Dr. F. Pascher, Bonn. Acetylations were effected by allowing the solution of the hydroxylic compound in excess of acetic anhydride and twice the volume of pyridine to stand overnight followed by removal of solvents *in vacuo*. Petroleum ether refers to the fraction boiling at 66–75°.

Calcd. for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63; O, 24.21; mol. wt., 264; 3 C-CH_3, 17.04. Found: C, 68.10; H, 7.63; O, 24.69; mol. wt., 260; C-CH_3, 15.51. Spectral properties included: λ_{max} 233 and 319 mμ (ε 13,200 and 3600); ν_{max} (cm.$^{-1}$) 3440 (s), 3000 (w), 2940 (w), 2890 (w), 1706 (s), 1653 (w), 1610 (s), 1105 (s), and 1038 (s); τ 3.55 (singlet, olefinic H); 5.3 (broad peak, $-CH_2-(OH)$); 6.43 (singlet, OH); 6.53 (singlet, $-CH_2-(OH)$); 8.0 (broad peak, 2 OH); 8.32, 8.65, 8.82 (all singlets, 3 $-CH_3$); 8.95–9.7 (multiplet, about 4 cyclopropane H; the exact number was not clear because of overlap by the methyl signals); $[\phi]_{589}$ −358° (*c* 1.00, methanol).

The *diacetate* crystallized readily from petroleum ether as rods, m.p. 99–100°; λ_{max} 227, 243 infl., and 313 mμ (ε 12,900, 11,100, and 3400); ν_{max} (Nujol) (cm.$^{-1}$) 3550, 1736, 1698, 1664, and 1605; τ 3.53 (singlet, olefinic H); 4.13 (singlet, $-CH-(OAc)$); 5.94 (AB quartet, J_{AB} = 11 c.p.s., δ_{AB} = 0.16 p.p.m.); 6.67 (singlet, OH); 7.88, 7.97 (singlets, 2 Ac); 8.47, 8.62, 8.87 (all singlets, 3 $-CH_3$); and 8.8–9.7 (multiplet, about 4 cyclopropane H).

Anal. Calcd. for $C_{19}H_{24}O_6$: C, 65.50; H, 6.94; 2 Ac, 24.71. Found: C, 65.45, H, 7.15; Ac, 25.43.

The *oxime* was obtained by refluxing a solution of XV (264 mg.), hydroxylamine hydrochloride (140 mg.), and sodium acetate (544 mg.) in ethanol (20 ml.) for 2 hr. The solution was then concentrated and cooled, giving crystals which had m.p. 212–215° dec. after recrystallization from methanol–water.

Anal. Calcd. for $C_{15}H_{21}O_4N$: C, 64.49; H, 7.58; N, 5.02. Found: C, 64.40; H, 7.82; N, 5.18.

Illudin-M (XVI). This was not as soluble as XV in polar organic solvents. On recrystallization from ethanol–water it had m.p. 128–130°; λ_{max} 228 and 318 mμ (ε 13,900 and 3600); ν_{max} (cm.$^{-1}$) 3400 (s), 1695 (s), 1661 (w), 1595 (s), 1381 (w), 1361 (w), 1030 (s); τ 3.47 (singlet, olefinic H); 5.62 (singlet, $-CH-(OH)$); 6.45 (singlet, OH); 8.33, 8.67, 8.81, 8.92 (all singlets, 4, $-CH_3$); and 8.4–9.7 (multiplet, cyclopropane H). Again, the exact number was not clear because of overlap by the methyl signals. The second OH probably gives a signal near this region. The integral curve gave a proton count of 20); $[\phi]_{589}$ −284° (*c* 0.42, methanol).

Anal. Calcd. for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12; O, 19.33; mol. wt., 248. Found: C, 72.64; H, 7.77; O, 19.28; mol. wt. (Rast), 241.

The *monoacetate* on recrystallization from petroleum ether had m.p. 75–76°; λ_{max} 226, 243 infl., and 312 mμ (ε 13,200, 11,800, and 3600); ν_{max} (Nujol) (cm.$^{-1}$) 3510, 1733 (sh), 1724, 1704, 1664, and 1605; τ 3.47 (singlet, olefinic H); 4.33 (singlet, $-CH-(OAc)$); 6.59 (singlet, OH); 7.92 (singlet, Ac); 8.47, 8.64, 8.82, 8.90 (all singlets, 4 $-CH_3$); and 8.9–9.7 (multiplet, 4 cyclopropane H).

Anal. Calcd. for $C_{17}H_{22}O_4$: C, 70.34; H, 7.59; O, 22.07; 1 Ac, 14.83. Found: C, 70.50; H, 7.42; O, 21.75; Ac, 19.06.

Conversion of Illudin-S to the Indenols I–IV. A. A solution of illudin-S in methanol was stirred with palladized charcoal (5%) in the presence of hydrogen at room temperature and pressure. Absorption of about 1.5–1.7 moles occurred quickly, then it slowed considerably. In a typical experiment, 500 mg. of XV absorbed 65 ml. of H_2 (1.6 moles) in 6 min. Further treatment for 24 hr. resulted in uptake of about 1 mole more. The noncrystalline product isolated after approximately 6 min. had λ_{max} 280 mμ; it was dissolved in very dilute ethanol, a drop of dilute sulfuric acid was added, and steam was passed through the heated solution. Crystalline material (white needles, 60 mg.) formed in the steam distillate, and in the reaction flask there remained an emulsion which partly crystallized on cooling. The steam-volatile material I on recrystallization from ethanol–water or on sublimation (0.05 mm., 70°) had m.p. 130–132°. It dissolved readily in dilute alkali; λ_{max} 266, 300, and 311 mμ (ε 9700, 2800, and 2600), strong absorption at 220 mμ; ν_{max} (Nujol) (cm.$^{-1}$) 3360, 1626, 1608, and 1580; τ 3.48 (quartet, *J* = 1.5 c.p.s., olefinic H); 5.7 (very broad peak, OH); 6.83 (broad singlet, cyclic $-CH_2-$); 7.33 (quartet, *J* = 7.5 c.p.s., $(Ar)-CH_2-(CH_3)$); 7.72, 7.79 (singlets, 2 aromatic $-CH_3$); 7.89 (broad singlet, olefinic CH_3); and 8.92 (triplet, *J* = 7.5 c.p.s., $(CH_2)-CH_3$).

Anal. Calcd. for $C_{14}H_{18}O$: C, 83.12; H, 8.97; mol. wt., 202. Found: C, 83.34; H, 8.82; mol. wt. (Rast), 191.

The *acetate* of I on recrystallization from petroleum ether had m.p. 92–93°; ν_{max} (Nujol) 1757 cm.$^{-1}$.

Anal. Calcd. for $C_{16}H_{20}O_2$: C, 78.65; H, 8.25; O, 13.10. Found: C, 78.94; H, 8.33; O, 12.88.

The *methyl ether* of I was prepared by adding excess sodium hydroxide solution (0.1 *N*) portionwise to a stirred solution of I in acetone and methyl sulfate. It had m.p. 34–35°.

The partly crystalline residue from the steam distillation had the same ultraviolet spectrum as I and it gave 100 mg. of the indenol II. This on recrystallization from ethanol–water had m.p. 147–149°; λ_{max} 266, 300, and 311 mμ (ε 9400, 2700, and 2600); τ 3.45 (quartet, *J* = 1.5 c.p.s., olefinic H); 5.87 (singlet, OH); 6.63 (singlet, OCH_3); 6.83 (broad singlet, cyclic $-CH_2-$); 6.3–7.25 (multiplet, partly obscured by the OCH_3 and cyclic $-CH_2-$, $(O)-CH_2-CH_2-(Ar)$); 7.70, 7.75 (singlets, 2 aromatic $-CH_3$); and 7.88 (broad singlet, olefinic CH_3).

Anal. Calcd. for $C_{15}H_{20}O_2$: C, 77.55; H, 8.68; O, 13.77; 1 OMe, 13.36. Found: C, 76.89; H, 8.72; O, 14.53; OMe, 13.04.

The *acetate* of II on recrystallization from petroleum ether had m.p. 91–92°; ν_{max} (Nujol) 1751 cm.$^{-1}$.

Anal. Calcd. for $C_{17}H_{22}O_3$: C, 74.42; H, 8.08; O, 17.50. Found: C, 76.71; H, 7.94; O, 15.40.

The *methyl ether* of II prepared in the usual way had m.p. 54–56° on recrystallization from ethanol–water.

When the hydrogenation of XV was carried out in ethanol and the noncrystalline product treated with acid, two compounds were isolated. One was the indenol I, the other an indenol $C_{16}H_{22}O_2$ (III) obtained in approximately the same yield as II. It had m.p. 121–123° after recrystallization from benzene; τ 3.48 (quartet, *J* = 1.5 c.p.s., olefinic H); 5.38 (singlet, OH); 6.48 (quartet, *J* = 7 c.p.s., $(O)-CH_2-(CH_3)$); 6.85 (broad singlet, cyclic $-CH_2-$); 6.25–7.2 (partly obscured multiplet, $(O)-CH_2-CH_2-(Ar)$); 7.7, 7.75 (singlets, 2 aromatic $-CH_3$); 7.87 (broad singlet, olefinic $-CH_3$); and 8.78 (triplet, *J* = 7 c.p.s., $(O-CH_2)-CH_3$).

Anal. Calcd. for $C_{16}H_{22}O_2$: C, 78.01, H, 9.00; O, 12.99; mol. wt., 246. Found: C, 77.76; H, 8.74; O, 13.38; mol. wt. (Rast), 274.

The *acetate* of III on recrystallization from petroleum ether had m.p. 91–94°; ν_{max} 1751 cm.$^{-1}$.

Anal. Calcd. for $C_{18}H_{24}O_3$: C, 74.97; H, 8.39; O, 16.64. Found: C, 74.62; H, 8.31; O, 16.82.

The *methyl ether* of III on recrystallization from methanol–water had m.p. 59–61°.

B. A solution of illudin-S (500 mg.) in methanol (30 ml.) was refluxed with zinc dust (2.5 g.) and dilute sulfuric acid–water (2:1 v./v.; 0.5 ml.) added. After 30 min. more acid (0.5 ml.) was added. Aliquots of solution were removed at intervals and the ultraviolet spectra taken. They showed that within 5 min. the dienone chromophore had disappeared and there was present instead the chromophore of the indenol. The intensity of the indenol absorption increased somewhat during the next 30 min. After 1 hr. the liquid was filtered off from the zinc, concentrated to half its volume, and diluted with water, giving a white precipitate. After standing overnight in the refrigerator, the precipitate (crystalline) was collected, washed with water, and dried, giving 300 mg. (68% yield), m.p. 130–140°. Recrystallization from benzene raised the melting point to 147–149°. It was identical (mixture melting point, infrared spectrum) with II. To the filtrate obtained after separation of the crude indenol was added dimedone (1 g.) dissolved in ethanol (12 ml.). A crystalline precipitate of formaldehyde dimethone formed gradually (40 mg.). It was identified by comparison with authentic material.

Repetition of the experiment with (1) ethanol instead of methanol gave III in similar yield. (2) Constant boiling hydrobromic or hydriodic acid (two 0.5-ml. portions) instead of sulfuric acid gave I. In this experiment granular zinc (30 mesh) was used instead of zinc dust. (3) Concentrated hydrochloric acid (two 0.5-ml. portions) and granular zinc gave the chlorinated indenol IV (~68%). On recrystallization from ethyl acetate–petroleum ether or on sublimation (0.1 mm., 120°) IV had m.p. 144–146°; λ_{max} 266, 302, and 312 mμ (ε 9700, 3000, and 2900), strong absorption at 220 mμ; ν_{max} (cm.$^{-1}$) 3400, 1623, 1608, and 1575.

Anal. Calcd. for $C_{14}H_{17}ClO$: C, 71.03; H, 7.19. Found: C, 71.94; H, 7.35.

The *acetate* of IV on recrystallization from petroleum ether had m.p. 130–132°; λ_{max} 263, 292, and 304 mμ (ε 12,300, 1700, and 1300); ν_{max} 1760 cm.$^{-1}$; τ 3.5 (quartet, J = 1.5 c.p.s., olefinic H); 6.86 (singlet, cyclic $-CH_2-$); 6.25–7.1 (A_2B_2 multiplet, (Cl)–CH_2–CH_2–(Ar)); 7.68 (singlet, 2 aromatic CH_3); 7.87 (singlet, acetate); 7.89 (singlet partly overlapping the acetate signal, olefinic CH_3).

Anal. Calcd. for $C_{16}H_{19}ClO_2$: C, 68.92; H, 6.87; Cl, 12.73; O, 11.5. Found: C, 68.38; H, 6.80; Cl, 12.68; O, 12.73.

The *methyl ether* of IV on recrystallization from methanol had m.p. 88–89°.

Anal. Calcd. for $C_{15}H_{19}ClO$: C, 71.87; H, 7.63; O, 6.38; 3 C–CH_3, 17.94; 1 OMe, 12.36. Found: C, 72.29; H, 7.62; O, 6.87; C–CH_3, 16.75; OMe, 12.96.

Reactions of Indenols I–IV. A. Catalytic Hydrogenation. The indenol (200 mg.) in ethanol (20 ml.) was stirred with palladized charcoal (150 mg., 30%) under hydrogen. The uptake in all cases was 1 mole. The product from I on recrystallization from methanol–water or sublimation (0.1 mm., 70°) had m.p. 101–103°.

Anal. Calcd. for $C_{14}H_{20}O$: C, 82.30; H, 9.87; O, 7.83. Found: C, 82.26; H, 9.63; O, 8.10.

The product from III on recrystallization from petroleum ether had m.p. 82–83°; τ 5.53 (singlet, OH); 6.47 (quartet, J = 7 c.p.s., (CH_2O)–CH_2–(CH_3)); 6.2–7.8 (multiplet, O–CH_2–CH_2–(Ar), 2 cyclic CH_2, –CH–(CH_3)); 7.76, 7.80 (singlets, 2 aromatic CH_3); and 8.77 (triplet, J = 7 c.p.s., CH_3–(CH_2), CH_3–(CH)).

Anal. Calcd. for $C_{16}H_{24}O_2$: C, 77.38; H, 9.74; O, 12.88. Found: C, 77.46; H, 9.84; O, 13.07.

The product from IV after sublimation (0.1 mm., 90°) had m.p. 101–103°.

Anal. Calcd. for $C_{14}H_{19}ClO$: C, 70.44; H, 7.97; Cl, 14.86; O, 6.71. Found: C, 71.25; H, 7.74; Cl, 13.99; O, 7.11.

B. Reaction with Tetrachloro-1,2-benzoquinone. The methyl ether of I (100 mg.) and the quinone (107 mg.) dissolved in benzene (10 ml.) were heated under N_2 to reflux temperature (causing the dark brown solution to lighten rapidly) and kept there for 1 hr. The pale yellow solution was then filtered through a column of alumina and the benzene was removed leaving an oil (V, 170 mg.) which soon crystallized. On recrystallization from ethyl acetate V had m.p. 163–165°; λ_{max} 295 infl. and 303 mμ (ε 3200 and 4000); ν_{max} 1425 cm.$^{-1}$ (the most intense peak in the spectrum).[4]

Anal. Calcd. for $C_{21}H_{20}Cl_4O_3$: C, 54.55; H, 4.36; Cl, 30.7; O, 10.49. Found: C, 54.61; H, 4.22; Cl, 31.33; O, 9.89.

An adduct was obtained from I itself under the same conditions. It had m.p. 244–248°.

C. Ozonization. A solution of the methyl ether of II (500 mg.) in ethyl acetate (20 ml.) was cooled in a Dry Ice–acetone bath and ozone passed through it for 1 hr. until all of the ether had reacted (ultraviolet spectrophotometric control). The pale blue solution was allowed to warm, water was added, and the liquids were heated on the steam bath to remove the ethyl acetate. An oily suspension remained which set to a gum on cooling. The water was decanted and the gum dissolved in benzene and filtered through a column of silica gel. Removal of the solvent gave the ozonide (VI, 450 mg.) which on recrystallization from ethanol had m.p. 95–96°; λ_{max} 274 and 283 mμ (ε 1100 and 1100).

Anal. Calcd. for $C_{16}H_{22}O_5$: C, 65.29; H, 7.53; O, 27.18; 1 OMe, 21.1; mol. wt., 294. Found: C, 65.93; H, 7.57; O, 26.75; OMe, 21.54; mol. wt. (Rast), 266.

A methanolic solution of VI consumed 1.02 moles of periodic acid overnight.

In another experiment VI (300 mg.) dissolved in acetic acid–water (2 ml.; 1:1 v./v.) was treated with chromium trioxide (1 g.) in the same solvents (10 ml.). Next morning most of the solvent was removed *in vacuo* and water added, giving an oily suspension which crystallized on standing (60 mg.). On recrystallization from methanol, the compound VII had m.p. 88–90°; λ_{max} 248 and 300 mμ (ε 9600 and 3400),

strong absorption at 220 mμ (addition of a drop of alkali gave a yellow solution, λ_{max} 251, 323, and 410 mμ (ε 13,700, 12,000, and 12,000). Acidification then gave a colorless solution, λ_{max} 250, 282, and 352 mμ. After 2 hr. this had changed back to the original spectrum); ν_{max} 1770 and 1733 cm.$^{-1}$; τ 4.3 (singlet, (O)–CH–(CO)); 6.2 (singlet, aromatic OCH_3); 6.6 (singlet, aliphatic OCH_3); 6.5–6.9 (multiplet, (O)–CH_2–CH_2–(Ar)); 7.3 (singlet, CH_3–(C=O)); 7.65 and 7.9 (singlets, 2 aromatic CH_3).

Anal. Calcd. for $C_{16}H_{20}O_5$: C, 65.74; H, 6.90; O, 27.37; 2 OMe, 21.21. Found: C, 66.09; H, 6.82; O, 27.01; OMe, 21.18.

Hydrogenation of Illudin-M (XVI). A solution of illudin-M (500 mg.) in methanol behaved in the same way as illudin-S on catalytic hydrogenation with palladized charcoal. The product (from *ca.* 1.5 moles uptake) was partly crystalline. The crystalline material VIII (230 mg.) which separated from a concentrated solution of the product in methanol had m.p. 163–165° after recrystallization from ethyl acetate–petroleum ether; λ_{max} 280 infl., 286 mμ (ε 1500 and 1700), strong absorption at 220 mμ; ν_{max} 3340, 3230, and 1588 cm.$^{-1}$; τ 5.4 (broad singlet, OH and (O)–CH–(Ar)); 6.62 (singlet, OCH_3); 6.4–7.5 (multiplet, (O)–CH_2–CH_2–(Ar) and cyclic –CH_2–); 7.65, 7.77 (singlets, 2 aromatic CH_3); 8.5 (broad peak, OH); 8.76 and 8.98 (singlets, *gem*-dimethyl).

Anal. Calcd. for $C_{16}H_{24}O_3$: C, 72.69; H, 9.15; O, 18.16; 3 C–CH_3, 17.05. Found: C, 72.63; H, 8.85; O, 18.78; C–CH_3, 15.38.

The *diacetate* on recrystallization from petroleum ether had m.p. 79–81°; ν_{max} 1760 and 1733 cm.$^{-1}$; $[\phi]_{589}$ −196° (*c* 0.97, methanol).

Anal. Calcd. for $C_{20}H_{28}O_5$: C, 68.94; H, 8.10; O, 22.96; 2 Ac, 24.74. Found: C, 69.03; H, 8.33; O, 22.90; Ac, 22.32.

When a solution of VIII in acetic acid was refluxed for 2 hr. a monoacetate was formed which had m.p. 105°; ν_{max} 3413 and 1736 cm.$^{-1}$ (saturated acetate).

Hydrogenolysis of VIII. The diol VIII (300 mg.) in methanol was stirred with palladized charcoal (150 mg., 30%) under hydrogen; absorption of 1.0 mole took place. The product isolated in the usual way had m.p. 123–125° after recrystallization from ethyl acetate–petroleum ether; ν_{max} 3370 and 1587 cm.$^{-1}$; τ 5.60 (singlet, OH); 6.62 (singlet, OCH_3); 6.3–7.3 (multiplet, (O)–CH_2–CH_2–(Ar)); 7.30, 7.37 (singlets, 2 cyclic –CH_2); 7.78, 7.86 (singlets, 2 aromatic –CH_3); and 8.82 (singlet, *gem*-dimethyl).

Anal. Calcd. for $C_{16}H_{24}O_2$: C, 77.37; H, 9.74; O, 12.88; 1 OMe, 12.5. Found: C, 77.15; H, 9.43; O, 13.49; OMe, 12.72.

The *acetate* purified by sublimation (0.1 mm., 110°), had m.p. 56–58°; ν_{max} 1760 cm.$^{-1}$.

Reduction of XV and XVI with Sodium Borohydride. Sodium borohydride (50 mg.) was added to a solution of illudin-M (XVI, 500 mg.) in methanol (10 ml.), and the solution was allowed to stand for 1 hr. Acetic acid (0.4 ml.) was added, then the methanol was removed *in vacuo* and water added causing precipitation of some material. The mixture was made alkaline with sodium bicarbonate and extracted with ether. The extract was dried (Na_2SO_4) and the ether evaporated leaving the crystalline triol, XI (450 mg.), which on recrystallization from ethyl acetate–petroleum ether had m.p. 142–144°; λ_{max} 256 (ε 22,400); ν_{max} 3400 and 1656 cm.$^{-1}$; τ 4.37 (broad singlet, olefinic H); 5.63, 5.78 (2 broad peaks, 2 –CH–(OH)); 7.85 (very broad peak, 3 OH); 8.41 (singlet, olefinic CH_3); 8.9 (singlet, 2 –CH_3); 8.95 (singlet, –CH_3), 8.9–9.7 (multiplet, about 4 cyclopropane H); $[\phi]_{589}$ −101° (*c* 1.0, methanol).

Anal. Calcd. for $C_{15}H_{22}O_3$: C, 72.01; H, 8.80; O, 19.2. Found: C, 71.81; H, 8.49; O, 19.36.

The *diacetate* on recrystallization from petroleum ether melted at 66–68°, solidified on further heating, and melted again at 113–115°; ν_{max} (cm.$^{-1}$) 3500, 1754, 1715, and 1656; τ 4.38 (broad singlet, olefinic H); 4.50, 4.55 (broad singlets, 2–CH–(OAc)); 7.83, 7.95 (singlets, 2 Ac); 8.05 (singlet, OH); 8.62 (singlet, olefinic CH_3); 8.87 (singlet, 2 CH_3); 9.03 (singlet, CH_3); and 9.0–9.7 (multiplet, about 4 cyclopropane H).

A *monoacetate* of XI was obtained by similar reduction of illudin-M acetate with sodium borohydride. On recrystallization from petroleum ether it had m.p. 92–94°; ν_{max} 3440 and 1653 cm.$^{-1}$.

Illudin-S (XV) was allowed to react similarly with sodium borohydride but the product (λ_{max} 256 mμ) was not obtained crystalline.

Cleavage of XV and XVI with Sodium Metaperiodate. A solution of XV (500 mg.) in water (5 ml., warm) was added to one of sodium metaperiodate (500 mg.) in water (5 ml.). The resulting solution was kept overnight, then extracted several times with ethyl acetate and the extract dried with Na_2SO_4. Removal of the solvent left XII (430 mg.), which on recrystallization from ethyl acetate had m.p. 192–194° dec. It effervesced vigorously with sodium bicarbonate solution; λ_{max} 226 and 259 mμ (ε 7500 and 7900); ν_{max} (cm.$^{-1}$) 3400, 3200, 2750, 2630, 1710, 1684, 1650, and 1600; $[\phi]_{589}$ +105° (*c* 0.505, methanol).

Anal. Calcd. for $C_{15}H_{20}O_5$: C, 64.27; H, 7.19; O, 28.54. Found: C, 64.24; H, 6.55; O, 28.65.

A solution of XVI (400 mg.) in methanol (3 ml.) and water (5 ml.) was added to one of sodium metaperiodate (800 mg.) in water (5 ml.) and methanol (3 ml.). The resulting solution was kept overnight, then evaporated to dryness *in vacuo* and the residue extracted with ethyl acetate. Removal of the solvent left XIII (350 mg.), which after repeated recrystallization from ethyl acetate had m.p. 214–215.5°; λ_{max} 227 and 259 mμ (ε 8600 and 8600); ν_{max} (cm.$^{-1}$) 3420, 2690, 2600, 1724, 1684, 1650, and 1590; $[\phi]_{589}$ +155° (*c* 0.615, methanol).

Anal. Calcd. for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 67.75; H. 7.56.

Compounds XII and XIII were also obtained by adding a slight excess of a solution of lead tetraacetate in chloroform to one of XV or XVI, in chloroform, and allowing them to stand overnight. The products were not crystalline, but on seeding with crystalline material from the periodate experiments they crystallized completely.

Cleavage of XI with Sodium Metaperiodate. A solution of XI (610 mg.) in ethyl acetate (25 ml.) was stirred vigorously with one of sodium metaperiodate (1.0 g.) in water (25 ml.) for 1 hr. The organic layer was separated, washed with water, and dried (Na_2SO_4). Removal of the ethyl acetate left XIV (400 mg.) which on recrystallization from ethyl acetate–petroleum ether

had m.p. 102–103°; λ_{max} 228 and 286 mμ (ε 12,100 and 4400); ν_{max} (Nujol) (cm.$^{-1}$) 3425, 1678 (α,β-unsaturated aldehyde and cyclopropyl ketone), 1658 (sh), and 1572; τ 0.3 (singlet, -CHO); 3.22 (singlet, olefinic H); 5.70 (broad peak, -CH-(OH)); 7.58 (singlet, OH); 7.80 (singlet, CH_3-(C=O)); 7.92 (singlet, olefinic $-CH_3$); 8.80, 8.98 (singlets, *gem*-dimethyl); 8.65, 9.2 (pair of multiplets symmetrical about their midpoint, A_2B_2 spectrum, 4 spirocyclopropane H).

Anal. Calcd. for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12; O, 19.33; mol. wt., 248. Found: C, 72.62; H, 8.21; O, 19.75; mol. wt. (Rast), 265.

Under similar conditions the monoacetate of XI, m.p. 92–94° (λ_{max} 256 mμ), was allowed to react, though more slowly, with sodium metaperiodate, giving a cleavage product (λ_{max} 228 and 280 mμ); ν_{max} ($CHCl_3$) 1733 and 1698 cm.$^{-1}$.

Cleavage of XI with Manganese Dioxide. The triol XI (50 mg.), dissolved in chloroform (5 ml.), was stirred with activated manganese dioxide (0.5 g.)[13] for 3 hr. The mixture was filtered and the chloroform removed from the filtrate leaving a gum (λ_{max} 228 and 286 mμ) which crystallized completely on seeding with a crystal of XIV.

The monoacetate of XI, m.p. 92–94°, on similar treatment gave the same product as was obtained by cleavage with sodium metaperiodate. Illudin-M was unaffected by prolonged treatment (24 hr.) with activated manganese dioxide.

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The Hydrolysis of Vitamin B_{12}. Studies with Model Amides

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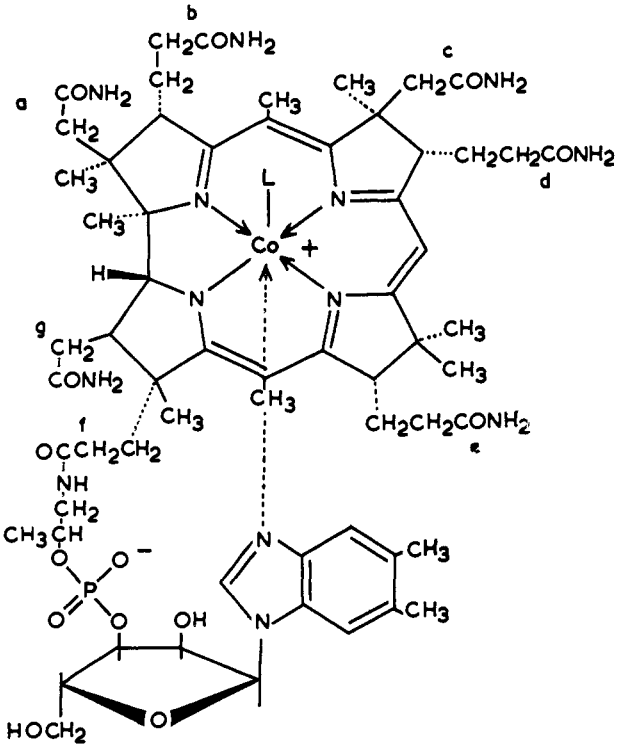
The suitability of certain cyclopentylacylamides as models for the steric situations of the amide groups in vitamin B_{12} is discussed. The syntheses of (1-methylcyclopentyl)acetamide, β-(1-methylcyclopentyl)propionamide, and β-(trans-2,2,3-trimethylcyclopentyl)propionamide are described, and the rates of hydrolysis of these and other model amides in aqueous hydrochloric acid–dioxane at 50° are recorded. Aquocobalamin has been hydrolyzed under identical conditions; comparison of these results with the model kinetics supports the earlier suggestion that steric factors have an important influence in the hydrolysis of vitamin B_{12}.

Introduction

The seven amide functions, two phosphate ester linkages, and ribosylamine group of vitamin B_{12} (I) lead to a complex fragmentation pattern when the vitamin is hydrolyzed.[1] The ribose–benziminazole bond, in fact, resists cleavage except under the most vigorous conditions, while methods which are fairly specific for the hydrolysis of the aminopropanol–phosphate linkage (leading to cobinamide) have been developed.[1,2] The present concern is the hydrolysis of the amide groups, a process which has been followed in detail[1] by the electrophoresis of the red acidic products. From this work it emerged that a considerable variation existed in the ease of hydrolysis of the amide groups; under mild acidic conditions (*e.g.*, 0.1 *N* HCl, room temperature, several days) three amide groups were cleaved to give mixtures containing three monobasic acids, three dibasic acids, and one tribasic acid, all of which retained the nucleotide. At the other extreme were two amide groups the hydrolysis of which required much more vigorous conditions (2 *N* HCl, 100°, 4 hr.).

A consideration of structure I suggests two major



I, L = CN, vitamin B_{12} (cyanocobalamin)
Ia, L = H_2O, aquocobalamin

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