iron on the rate of oxidation of ascorbic acid but with dialuric acid the effect is reversed. These observations are consistent with the suggestion that copper acts more effectively than iron as a catalyst for the oxidation of the enediol configuration (ascorbic acid); conversely, iron may act more effectively as a catalyst for the oxidation of the α-keto-hydroxy configuration (dialuric acid).

Levy[10] has concluded that ascorbic acid exists in its ketonic form in acid solution and Huelin and Stephens[11] have observed that the relative catalytic effects of copper and iron on the oxidation of ascorbic acid are reversed as the *p*H of the solution is decreased from 3.0 to 0.4. The specificity of copper as a catalyst for the enediol group has been discussed by Dodds.[12]

(10) L. F. Levy, *Nature*, **152**, 693 (1943).
(11) F. E. Huelin and I. M. Stephens, *ibid.*, **158**, 703 (1946).
(12) M. L. Dodds, *Arch. Biochem.*, **18**, 51 (1948).

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A New Synthesis of 4a(H)-Dodecahydrobenzo(c)-quinolizine

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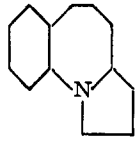
Of the four racemic modifications possible in 4a-(H)-dodecahydrobenzo(c)quinolizine, Clemo and co-workers[2] have reported the preparation of two, "A" and "B." Subsequent work by Leonard and Wildman[3] has shown that the precursor to Clemo's compound "A," 1-keto-6,7-hexahydrobenzoquinolizidine, had undergone disproportionation of two rings during a Clemmensen reduction to give a structure shown by I.



I

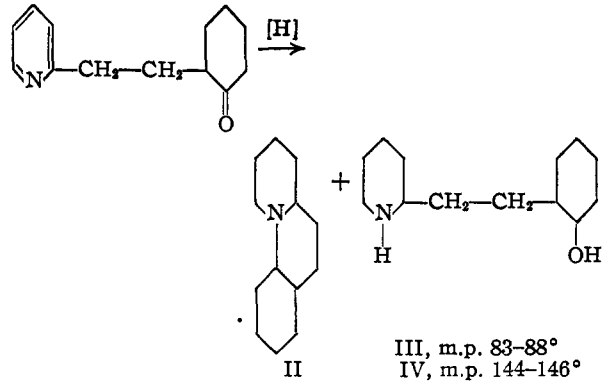
Our compound has physical constants differing from those of Clemo's compound "B" and is, therefore, presumed to be a heretofore unknown racemate.

Results and Discussion

Reduction of 2-(2-ethylpyridyl)-cyclohexanone in aqueous HCl over PtO_2 gave three products, of which one IV, was formed to the extent of about one per cent. under all temperatures employed. The ratio of II and III formed was found to be a function of the temperature, with II predominating at higher temperatures at the expense of III.

The uncyclized alcohol III could not be dehydrated to give the cyclic 4a(H)-dodecahydrobenzo-(c)quinolizine (II) when subjected to the same conditions prevalent at the formation of II. It is

(1) Abstracted from a thesis submitted by Warren E. Solodar in partial fulfillment of the Master of Science degree, Stevens Institute of Technology.
(2) G. R. Clemo, J. G. Cook and R. Raper, *J. Chem. Soc.*, 1318 (1938).
(3) N. J. Leonard and W. C. Wildman, THIS JOURNAL, **71**, 3089 (1949).



possible that this racemic form of the alcohol III is not an intermediate in the formation of II.

Reaction of II with methyl iodide gave two methiodides which could not be completely separated by fractional crystallization. The partially separated fractions had melting points of 185–186° and 242–249° and both analyzed for the quaternary ammonium compound expected from 4a(H)-dodecahydrobenzo(c)quinolizine and one equivalent of methyl iodide. The fact that II forms a single, constant-melting picrate is taken as proof that it is a single racemic modification; the two methiodides represent a pair of diastereoisomers which owe their existence to the asymmetric nitrogen atom.

Experimental Part

2-(2-Ethylpyridyl)-cyclohexanone was prepared by the procedure of Levine and Wilt[4] in 38% yield, b.p. 137–143° (1 mm.), $n^{20}D$ 1.5311.

2-(2-Ethylpiperidyl)-cyclohexanol (IV).—Fifty-one grams (0.25 mole) of 2-(2-ethylpyridyl)-cyclohexanone was reduced in aqueous HCl at 55–60° over one gram of PtO_2 at 1500 p.s.i. of hydrogen. The theoretical uptake of hydrogen (4 equivalents) required 5 hours. The solution was decanted from the catalyst, made basic with aqueous alkali, and extracted with benzene. The benzene was evaporated, ether was added, and the mixture kept at 0° for 2 days. A precipitate of white crystals was filtered off (1.5 g., m.p. 107–112°) and recrystallized twice from ether, giving 0.52 g. of fine white needles, m.p. 144–146°.

Anal. Calcd. for $C_{13}H_{25}NO$: C, 73.88; H, 11.92; N, 6.63. Found: C, 73.70; H, 11.54; N, 6.82.

The *p*-nitrobenzoate (N-*p*-nitrobenzoyl), prepared in the usual manner, crystallized from ethanol in yellow prisms, m.p. 144–146°. An intimate mixture of it and the starting alcohol IV melted at 132–140°.

Anal. Calcd. for $C_{27}H_{31}N_3O_7$: C, 63.64; H, 6.13; N, 8.25. Found: C, 63.44; H, 6.03; N, 8.31.

4a(H)-Dodecahydrobenzo(c)quinolizine (II).—The ether filtrate from the filtration of IV above was evaporated and distilled, giving 38 g. (79%) of colorless liquid, b.p. 113–115° (1.5 mm.) and a small amount of higher boiling material, subsequently identified as compound III. Redistillation gave 30 g. of colorless liquid, b.p. 71–74° (0.5 mm.), $n^{24}D$ 1.5080.

Anal. Calcd. for $C_{13}H_{23}N$: C, 80.76; H, 11.99; N, 7.25. Found: C, 80.52; H, 12.25; N, 7.38.

The picrate, prepared in ether, crystallized from an ethanol–water mixture in fine yellow crystals, m.p. 178–180°.

Anal. Calcd. for $C_{19}H_{26}N_4O_7$: C, 54.02; H, 6.20; N, 13.26. Found: C, 54.45; H, 6.03; N, 13.67.

The methiodides were prepared by refluxing the base with excess methyl iodide in ethyl acetate for 0.5 hour. Repeated fractional crystallization from methanol–acetone mixtures gave two fractions which could not be purified to constant melting points.

Anal. Calcd. for $C_{14}H_{26}NI$: C, 50.15; H, 7.81; N, 4.18. Found: fraction a, m.p. 242–249°: C, 49.73; H, 7.58; N,

(4) R. Levine and M. H. Wilt, *ibid.*, **74**, 342 (1952).

4.74. Fraction b, m.p. 185–186°: C, 50.48; H, 7.94; N, 3.93.

2-(2-**Ethylpiperidyl**)-**cyclohexanol** (III).—The reduction of 2-(2-ethylpyridyl)-cyclohexanone was carried out as in the preparation of IV above, but at room temperature (50 hours). The reduction mixture was decanted from the catalyst, made basic with aqueous alkali, and extracted with chloroform. The chloroform was dried, evaporated, and the residue distilled, giving 57% of a thick, colorless liquid, b.p. 145–148° (2 mm.). This was crystallized from Skellysolve B, the crystals taken up in boiling ether, and set at 0° for 2 days. A small amount of IV was filtered off, and the ether filtrate evaporated. The residue was recrystallized from Skellysolve B, giving a 40% yield of white solid, m.p. 83–88°.

Anal. Calcd. for $C_{13}H_{25}NO$: C, 73.88; H, 11.92; N, 6.63. Found: C, 73.83; H, 12.14; N, 6.91.

The *p*-**nitrobenzoate** (N-*p*-**nitrobenzoyl**), prepared in the usual manner, crystallized from ethanol in pale yellow prisms, m.p. 158–160°.

Anal. Calcd. for $C_{27}H_{31}N_3O_7$: C, 63.64; H, 6.13; N, 8.25. Found: C, 63.96; H, 6.32; N, 8.30.

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Carotenoids in *Phycomyces*

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A marked effect on β-carotene production in *Phycomyces* may be shown by addition of β-ionone to cultures.[1] Recent experiments with purified α-ionone indicate that this isomer also enhances β-carotene production. By contrast, we reported that citral and pseudoionone had a slight effect on lycopene and that methylheptenone had no effect. This requires revision. Control and ionone-treated cultures, harvested 60 to 100 hours after inoculation on a glucose–yeast autolysate medium give extracts whose carotenoid spectrum is essentially that of β-carotene. Methylheptenone-treated cultures show weak pigmentation, when compared with controls, but the carotenoid spectrum is radically different. After chromatography of the crude petroleum ether extracts on MgO–SiO_2 columns and spectrophotometric estimation of the components, the following effects of methylheptenone may be shown: production of β-carotene is halved; the phytofluene content is increased 6- to 15-fold; ζ-carotene which is not demonstrable in control cultures grown under our conditions is found in significant amount, neurosporene is detected, and the lycopene content is also increased.

Culture conditions and procedures have already been described.[1] Methylheptenone, 20 μl., (Fritzsche Bros.) was applied to each culture at times varying from 6 to 27 hours after inoculation. To minimize adverse growth effects, the methylheptenone should be applied 12 to 24 hours after inoculation. If applied immediately germination of the spores is unduly delayed.

The following results are typical of several independent runs. Figures for the heptenone-treated cultures precede values for the controls, in μg. carotenoid per g. of dry mycelium, each value representing five plates: (1) cultures treated 24 hr. after inoculation, harvested in 60 hr., *vs.* controls; dry weights, 0.400, 0.573 g.; phytofluene 46, 3.7; β-carotene 118, 253; ζ-carotene 60, not detected. (2) Cultures treated 17.5 hr. after inoculation, harvested in 112 hr., *vs.* controls; dry weights 0.555, 0.582 g.; phytofluene 88.5, 5.8; β-carotene 195, 392; ζ-carotene 43.3, trace.

In no case was neurosporene detected in the controls, though present in the treated cultures. The lycopene zone was definitely more prominent in the treated cultures, but at best was still a minor component, not exceeding 5 to 10 μg./g.

The striking effects of methylheptenone are therefore three: reduction of the β-carotene, a marked increase in phytofluene and the appearance of ζ-carotene as a major constituent. Re-examination of the absorption curves from extracts of citral-treated cultures makes it apparent that they are intermediate between controls and methylheptenone-treated cultures. Loss in β-carotene is not so marked, nor is production of phytofluene so enhanced, under comparable conditions.[2] These qualitative interpretations are supported also by the observation that the fluorescence of the citral-culture extracts is intermediate in intensity. We cannot as yet comment on possible additional effects ascribable to pseudoionone.

We have hitherto been puzzled by failure to detect numerous minor components observed by Goodwin.[3] He obtained phytofluene yields of 70 to 109 μg. per 250 ml. culture solution (Table 9, ref. 2) after 9 days growth on a 3% glucose–0.2% asparagine medium, in the presence of diphenylamine. Our 5 plates, collectively containing 100 ml. medium, produced 49 μg. of phytofluene (run 2), from 0.555 g. of dry matter, in a total of 112 hr., when treated, compared with 3.4 μg. for the controls. It is clear that great variation may be anticipated in the proportions of the different carotenoids comprising the mixture.

(1) G. Mackinney, T. Nakayama, C. O. Chichester and C. D. Buss, THIS JOURNAL, **74**, 3456 (1952); **75**, 236 (1953).

(2) The culture response to citral is affected more by the mode of application than is the response to ionone.

(3) T. W. Goodwin, *Biochem. J.*, **50**, 550 (1952).

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The Reaction of Diphosphopyridine Nucleotide with Sodium Borohydride—A Correction and Extension

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It was previously observed[1] that diphosphopyridine nucleotide (DPN) was quantitatively reduced by sodium borohydride, in agreement with values obtained by reduction with sodium

(1) M. B. Mathews, *J. Biol. Chem.*, **176**, 229 (1948).