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.

Acknowledgments.—The authors wish to thank the Scientific Management of Hoffmann–La Roche, Inc., Nutley, N. J., for the use of their facilities during this study, Dr. Al Steyermark of the Microchemical Department at Roche, for the microanalyses reported in this paper, and Professor N. J. Leonard, for his comments.

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Carotenoids in *Phycomyces*

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RECEIVED JULY 29, 1953

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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RECEIVED JUNE 25, 1953

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).