A NEW BACTERIOXANTHOPHYLL, THE YELLOW PIGMENT OF SARCINA LUTEA.[1]

(Preliminary Report.)

By Teruko NAKAMURA.

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In recent years, inquiries into the chemical nature and biological significance of organic colouring matters have been made to a great extent, but very few pigments of microorganisms have been studied chemically. The chemical nature of most of the pigments in highly coloured bacteria is not well known, although its study would be of interest not only for the understanding of the biological rôle of the bacterial pigments themselves, but also for comparison in cases of pigments which are also found in higher plants.

The writer has studied the yellow pigment of *Sarcina lutea*[1] which is prevalent in air. Although the occurrence of carotenes in bacterial pigments

(1) After the completion of this manuscript, the author has found a note on the pigment of *Sarcina lutea*: E. Chargaff and J. Dieryck, *Naturwiss.*, **20** (1932), 872.

is generally accepted and moreover demonstrated by absorption spectra[2] and by feeding experiments[3] for the vitamin A activity, reports on the examination of carotenoids isolated as such from any bacterial growth are still meager.

The pale yellow pigmentation of *Sarcina lutea* with green fluorescence which is distinct from well known red or orange coloured carotenoids, e.g. lycopene or carotenes, seemed to furnish a new interesting material for study. The pigment was found to be carotenoidal, containing chiefly a xanthophyllic substance which gave ordinary lipochrome reactions and which revealed absorption maxima at 490, 460, and 433mμ in carbon bisulphide. The study of such hitherto undefined yellow pigment of microorganism is of interest in connection with the investigation of xanthophyllic matter in higher plants as they are conjectured to be the precurser of carotenes. The present paper is a preliminary report.

Experimental.

Isolation of the Pigment. The organism was grown on broth agar at 22°.[4] The medium was slightly alkaline (*p*H 7.2). A solid culture was preferred to a liquid medium because on agar it was found to grow more rapidly and also to produce more pigment. The organism reached maximum pigmentation in approximately 10 days, when the growth was scraped off the surface.

The pigment was collected as follows: The cultures were extracted with cold 98% methyl alcohol in the dark at room temperature, until the cellular material became almost colourless; the pigment was taken up as a clear yellow methyl alcoholic solution with green fluorescence. The alcoholic solution was then distilled to dryness in a stream of CO_2 gas at 5.° under a diminished pressure. The residue consisted of a greenish yellow matter. It was washed with a small quantity of methyl alcohol which dissolved the pigment leaving a great portion of a white residual matter undissolved. The latter was readily soluble in water and sparingly soluble in organic solvents.

The methyl alcoholic solution of the pigment was concentrated by distillation and was diluted with water until it contained 30% methyl alcohol, and the solution was extracted with ether. From the ether extract, the solvent was removed, leaving the crude pigment of greenish yellow colour, which was tested chemically and spectroscopically.

Solubility. Insoluble in H_2O, in aqueous acid (HCl), and alkaline solutions (NaOH), soluble in methyl alcohol, ethyl alcohol, ether, chloroform, carbon bisulphide, acetone, benzine, benzene, petroleum ether, and xylene. Among the above solvents it is most soluble in methyl alcohol and only slightly soluble in petroleum ether and benzine.

Colour Reactions. Blue colourations with conc. H_2SO_4, with $SbCl_3$, and with chloroacetic acid in chloroform. No colouration with 25% HCl, conc. HCl, HNO_3, acetic acid,

(2) V. Reader, *Biochem. J.*, **19** (1925), II, 1041.

(3) Carl A. Baumann, H. Steenbock, Mary A. Ingraham, and E. B. Fred, *J. Biol. Chem.*, **103** (1933), 339.

(4) On the improved method of cultivation, a report will be made by Mr. R. Ohtake.

and phosphoric acid. On addition of HCl or NaOH to the alcoholic solution no appreciable change was obs rved in the colour.

Oxidation and Reduction. The alcoholic solution was decolourized by Mg and HCl, and by H_2O_2 on standing.

Adsorption Tests. From the ether or benzine solution, the pigment could be adsorbed on several substances, e.g. Al_2O_3, kaolin, silica, fuller's earth, and "Faserton-erde" (Fränkel), but not on $CaCO_3$.

The elution of the adsorbed pigment, however, was difficult. Only in the cases of Al_2O_3 and "Faserton erde" the pigment could be separated in 1–5% solution of methyl alcohol in petroleum ether or benzine. This behavior is comparable with that of cryptoxanthine.(5)

Spectroscopic Measurements. With a hand spectroscope the following absorption maxima were read:

Solvent	Absorption maxima (mμ)		
Carbon bisulphide	490	460	(433)
Chloroform	475	445	(423)
Alcohol	467	436	(410)
Methyl alcohol	468	435	(408)
Ether	468	437	(413)
Benzine	470	440	

The third maximum placed in brackets was not distinct.

The spectrographic readings are lower than in any of the already identified carotenoids. Among similar compounds, the nearest values found in the literature are those of a short wave xanthophyll (468–469, 438–439 in alcohol),(6) β-xanthophyll of Tswett which is noted by Kuhn, and of canary xanthophyll(7) (472, 443, 418 in benzine). But as already mentioned the pigment of *Sarcina lutea* is not adsorbed on $CaCO_3$, while β-xanthophyll is.

Saponification. A solution of the pigment in methyl alcohol was saponified with alcoholic potash, the concentration of the alkali being 2.5%. It was kept at 50° for 2 hours in a stream of hydrogen. On expelling a portion of the alcoholic solvent by distillation, the pigment became soluble in water. From the aqueous solution it could not be extracted with ether. On acidifying, however, the pigment was taken up in ether. It was also possible to extract the pigment with ether after saturating the alkaline solution of saponification with a stream of CO_2.

Notes. Minute quantity of a yellow crystalline substance was obtained from benzine and acetone solutions of the pigment. Further work is in progress to obtain the pigment

(5) R. Kuhn and C. Grundmann, *Ber.*, **66** (1933), 1746.
(6) R. Kuhn and H. Brockmann, *Z. physiol. Chem.*, **206** (1932), 60.
(7) H. Brockmann and O. Völker, *Z. physiol. Chem.*, **224** (1934), 209.

in pure crystalline state by the method of chromatograph ;[8] thereby the pigment may be divided into components.

Summary.

The yellow pigment of *Sarcina lutea* was examined. It is a lipochromic substance with absorption maxima at 490, 460, and 433 mμ in carbon bisulphide apparently distinct from well known xanthophylls, the constitution of which had not been identified. Saponification showed the substance to be chiefly a xanthophyll-like ester. The work is being continued.

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R. Majima Research Laboratory,
The Institute of Physical and
Chemical Research.

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(8) R. Kuhn and H. Brockmann, *Z. physiol. Chem.*, **206** (1932), 41. A. Winterstein and G. Stein, *Z. physiol. Chem.*, **220** (1933), 247.