

In this calculation the fact that, for a weak concentration, oxygen-18 is almost completely combined in a mixed molecule $^{16}\text{O}^{18}\text{O}$ must be taken into account. The isotopic yield is then:

$$\frac{1}{2} \times \frac{(^{16}\text{O}^{18}\text{O})}{(^{16}\text{O}^{16}\text{O})}, \text{ that is, } \frac{1}{2} \times \frac{34}{32}$$

It should also be pointed out that, if the evaporated ozone has not been carefully eliminated from the fluorochloromethane solvent, the measurements from the mass spectrograph could be erroneous. The CFCl_3 molecule, when ionized, gives indeed radicals of masses 66 and 68. When they are twice ionized, they appear on the spectrogram on abscissa 33 and 34.

The separation coefficient has been found to be:

$$\alpha = \frac{(^{18}\text{O})_{\text{final}}}{(^{16}\text{O})_{\text{final}}} \frac{(^{16}\text{O})_{\text{initial}}}{(^{18}\text{O})_{\text{initial}}} = 1.07$$

α is thus greater than unity, which brings us to the conclusion that there is an oxygen-18 enrichment of the oxygen when subjected to the foregoing treatment for the preparation of concentrated ozone. A series of samples of the final oxygen, taken at different times during the evaporation of ozone, gives the same value for α . This value of α is comparable with that obtained in the process of isotope separation by diffusion of natural oxygen.

These preliminary results call for a more detailed theoretical and experimental examination to allow the contributions of each operation to the observed overall effect to be determined—ozonization of oxygen, dissolution, and evaporation of ozone. However, the final evaporation does not appear to play an effective part since the same result ($\alpha = 1.07$) is obtained from both head and tail samples.

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¹ Chretien, A., Servigne, M., and Mahieux, F., *Bull. Soc. Chim. France*, 49 (1960).

² Ogg, R. A., and Sutphen, W. T., *J. Chem. Phys.*, 2078 (1953).

³ Ogg, R. A., and Sutphen, W. T., *Disc. Farad. Soc.*, 47 (1954).

BIOCHEMISTRY

Discovery of a Pyrazine in a Natural Product: Tetramethylpyrazine from Cultures of a Strain of *Bacillus subtilis*

WHILE investigating products in cultures of a strain of *Bacillus subtilis*, we obtained a crystalline substance which sublimed very easily even at ordinary temperature and had the characteristic smell of fermented soybean.

After cultivating the bacillus for a few weeks in media with sucrose as a source of carbon, asparagine as a source of nitrogen and metal ions (magnesium, sodium, potassium, ferrous, manganese and cupric ions), the whole cultures were subjected to flash evaporation. Water was partly distilled off and residue salted out and extracted with as little ether

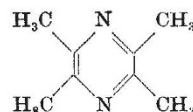
as possible, to avoid loss of the substance with the solvent on distillation. Ether was driven off on a water-bath and the residue dried with a small amount of benzene.

Purification was effected by sublimation. This gave a white crystalline substance melting at 87° C. and taking up moisture from the air very rapidly. The infra-red spectrum showed that the substance was comparatively simple and had no oxygen atom in the structure.

From its character and a rough carbon-hydrogen analysis, it was thought that foreign atoms, possibly nitrogen, would be present besides carbon and hydrogen. The presence of nitrogen was confirmed by preparing the picrate derivative. Preparation of the picrate made possible considerable progress in the isolation of this very volatile solid. After extraction with ether, the new substance was converted directly into picrate by addition of picric acid, and the yield was thereby increased about ten times that obtained by the sublimation procedure.

Carbon, hydrogen and nitrogen analysis of the picrate and the picrolonate indicated the molecular formula $\text{C}_8\text{H}_{12}\text{N}_2$.

All determinations, including melting points of the picrate and the free substance and the infra-red spectrum, are in agreement with the data for tetramethylpyrazine in the literature. It was confirmed that the crystalline substance is identical with an authentic sample of tetramethylpyrazine by mixed melting points and comparisons of their infra-red spectra.



This, we believe, is the second time that pyrazines have been found in natural products.

It is perhaps of some interest that tetramethylpyrazine was not produced in cultures containing peptone instead of asparagine as a source of nitrogen. Comparatively little is known about the biological and medical significance of pyrazines, but it is thought that they will have an important influence on living organisms, as a kind of diazine. Work on the biological activity of tetramethylpyrazine is being carried on by us.

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Purification of a New *b*-Type Cytochrome from Mung Bean (*Phaseolus aureus*) Seedlings

ON the basis of spectral observations, four *b*-type cytochromes (*b*, *b*₃, *b*₆, *b*₇) have been identified in higher plant tissues¹. Of these pigments, all of which are bound firmly to the cytoplasmic particles, only cytochrome *b*₃ has been extensively purified². In the course of an examination of the cytochromes in etiolated mung bean seedlings, we have isolated a soluble pigment which has the properties of a *b*-type cytochrome; it has an unusual α -band (reduced), with a peak at 555 and a shoulder at 559 μ . The