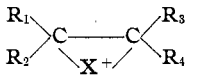
has, therefore, been postulated that the second step of the reaction (the addition of halide ion to the positive organic ion) takes place so rapidly that there is not sufficient time for rotation about the single bond to take place.

We would like to point out that free rotation about the C–C bond is *not* to be expected. If this structure is assumed, one of the orbitals of the $C^+$ must be completely empty. The X atom on the other hand has three orbitals occupied by pairs of electrons. This arrangement is such that a co-ordinate link will almost certainly be formed by the sharing of one of the pairs of electrons of the halogen with the unoccupied orbital of the carbon. Another possible structure of the ion is one in which the positive charge is on the halogen. The $X^+$, being isoelectronic with a member of the oxygen family, should show a valence of two, *i. e.*, it should form a structure of the ethylene oxide type




(2)

From an electronic viewpoint structures (1) and (2) are identical. The difference between the ionization potential of carbon (11.22 volts) and that of a halogen (*e. g.*, 11.80 volts for bromine) is so small that the actual structure of the ion is undoubtedly intermediate between (1) and (2). Since the two carbons in either structure are joined by a single bond and by a halogen bridge, free rotation is not to be expected.

If, however, $R_1$ and $R_3$ (or $R_2$ and $R_4$) are similarly charged groups (*e. g.*, $COO^-$) there may be sufficient repulsion between them to overcome the restraining force of the double linkage, and rotation to the opposite configuration may take place before the second step of the reaction occurs.

This second step, which may be the addition of either a halogen ion $X^-$ or some other atom or molecule, is probably a simple "three-atom" reaction of the type proposed by London,[6] and developed by Polanyi[7] and Olson.[8] In this case the new atom will approach one of the carbon atoms from the side opposite to the X atom already present. A bond to this carbon will be formed while the bond from the original X to the carbon is broken, with simultaneous neutralization of the charge of the ion. This process will always lead to *trans* addition, except in the previously mentioned case in which there are two like charged groups initially in the *cis* position.

With this modification the mechanism suggested by Robinson and by Bartlett and Tarbell explains all the existing data on the reactions of the halogens with ethylene linkages. The additions of bromine and chlorine to maleic and fumaric acids are very largely *trans*,[9] as the theory predicts. The additions of bromine and chlorine to fumarate ion are again predominantly *trans*, but the addition of bromine or chlorine to maleate ion, with its two negatively charged *cis*-carboxylate ions, is almost entirely *cis*.[10]

The difficulty of explaining the maintenance of configuration in the bromination of stilbene and isostilbene, which yield different methoxybromides and different dibromides[1] no longer exists if this structure of the intermediate ion is postulated. This is also true of the formation of the halo-beta-lactones from dimethylmaleic and dimethylfumaric acids.[2]

The authors wish to thank Prof. L. P. Hammett for his helpful discussions of this problem.

(6) London, *Z. Elektrochem.*, **35**, 552 (1929).

(7) Meer and Polanyi, *Z. physik. Chem.*, **B19**, 164 (1932); Bergmann, Polanyi, and Szabo, *ibid.*, **B20**, 161 (1933).

(8) Olson, *J. Chem. Phys.*, **1**, 418 (1933); Olson and Voge, THIS JOURNAL, **56**, 1690 (1934).

(9) McKenzie, *J. Chem. Soc.*, **101**, 1196 (1912).

(10) Terry and Eichelberger, THIS JOURNAL, **47**, 1067 (1925); Kuhn and Wagner-Jauregg, *Ber.*, **61**, 519 (1928).

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## A Fermentation Test for Vitamin $B_1$

BY ALFRED SCHULTZ, LAWRENCE ATKIN AND CHARLES N. FREY

Vitamin $B_1$ (Merck's natural-crystalline) exerts a powerful action on the rate of alcoholic fermentation. In the presence of a suitable sugar–salt buffer mixture as little as one gamma (0.000001 g.) of the vitamin may be detected.

Table I gives the results of a typical test.

TABLE I

Total volume in each case, 100 cc. (distilled water). Yeast for each, 1 g. commercial bakers' yeast (Fleischmann). Sugar, 3 grams Merck C. P. Dextrose, plus synthetic salt mixture and buffer. Temperature, 30°. Oscillations, 100 per minute.

| Natural crystalline vit. $B_1$ mg. | Cc. of gas in 3 hours |
|---|---|
| None | 185 |
| 0.001 | 215 |
| .005 | 305 |
| .010 | 350 |
| .040 | 395 |
| .100 | 405 |

It is natural to suppose that this phenomenon could be employed for vitamin $B_1$ assay. This has been done and when compared with assay by rat growth test the results so far have shown a very satisfactory concordance. We have been enabled to test synthetic vitamin $B_1$ (Merck "Betabion") through the courtesy of Merck & Co. The Betabion gives results scarcely distinguishable from the natural crystalline product.

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810 GRAND CONCOURSE
NEW YORK, N. Y. RECEIVED DECEMBER 22, 1936

## Debromination of Mono and Dibromocholestanone

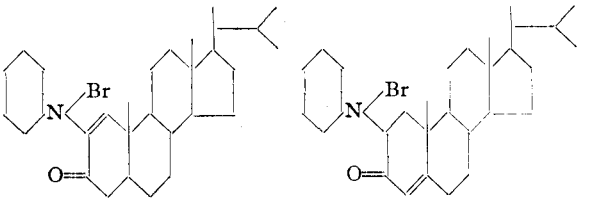
BY E. SCHWENK AND B. WHITMAN

We have been studying for some time the debromination of various sterol bromides, particularly those compounds in which the reaction would lead to unsaturation in the first ring. The appearance of several articles[1] dealing with this subject makes it seem desirable to publish some of our results now.

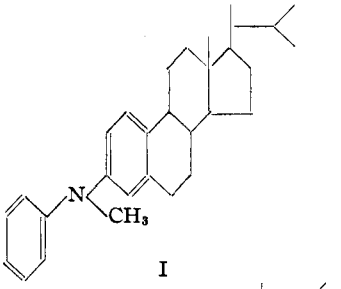
We have found that the nature of the reagent used to remove hydrogen bromide from bromo sterols has considerable influence on the course of reaction. It is thus possible to obtain several different reaction products from the same bromo compound.

Experiments with mono- and dibromocholestanone are illustrative of this point. From monobromocholestanone, by refluxing with pyridine, we obtained the same pyridinium compound that Butenandt and Wolff[2] reported. However, with dimethylaniline, monobromocholestanone yields mainly cholestanone. In this case the bromine has been replaced by hydrogen. This is the only example of such a reaction of which we are aware.

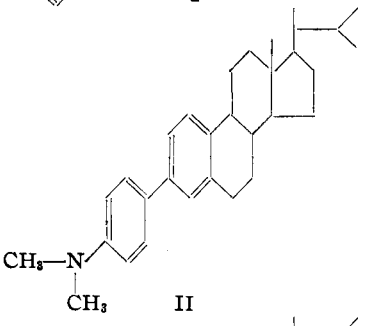
With dibromocholestanone pyridine yields an unsaturated pyridinium salt, arising very probably by splitting one bromine atom as hydrobromic acid and forming a salt with the other. For the dibromocholestanone, the 2,2,[1b] as well as the 2,-4,[1c] position for the bromine atoms has been discussed. Accordingly, the new pyridinium compound derived from dibromocholestanone would have one of the structures



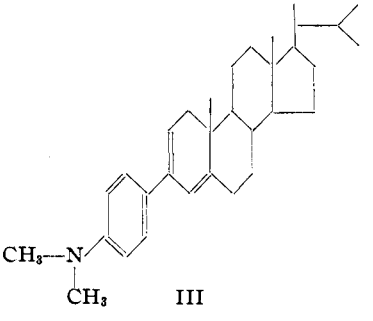

On refluxing dibromocholestanone with dimethylaniline, a substance was obtained which by analysis was found to contain only carbon, nitrogen and hydrogen, but no oxygen. The presence of nitrogen and absence of oxygen suggests that some condensation involving the keto group and the benzene ring has taken place. That such is the case is supported by the fact that this substance gives a beautiful wine-red coupling reaction with nitrodiazobenzene. This acid coupling is typical of an amine. The analytical results point to the substance being either $C_{33}H_{47}N$(I) or $C_{34}H_{49}N$(II) rather than $C_{35}H_{53}N$(III). In our opinion, formula II is the most probable. This would mean that ring A of the cholesterol has become aromatic.




I




II




III

The new substance is distinguished by the fact that its solution in acetic acid couples immediately

(1) (a) Butenandt and Wolff, *Ber.*, **68**, 2091 (1936); (b) Ruzicka, Bosshard, Fischer and Wirz, *Helv. Chim. Acta*, **19**, 1147 (1936); (c) Butenandt, Schramm, Wolff and Kudszus, *Ber.*, **69**, 2779 (1936).

(2) Butenandt and Wolff, *ibid.*, **68**, 2092 (1936).