zation of mescaline to 2-methyl-6,7,8-trimethoxy-1,2,3,4-tetrahydroisoquinoline as a result of attempts to prepare N,N-dimethylmescaline by the Clarke–Eschweiler procedure.

While the Pictet–Spengler[5,6] method of cyclization of phenethylamines to tetrahydroisoquinolines usually employs mineral acid, the conditions of acidity are not far removed from those in the usual Clarke–Eschweiler, especially if, as is often convenient with small quantities, the excess of formic acid is not minimized.

Since up to the present, the Pictet–Spengler had not been observed in the absence of strong acid except with phenolic amines[7] and a recent study of the Wallach reaction (of which the Clarke–Eschweiler is a special case) has shown that acidity is not required for it[8] the probability seemed high that homoveratrylamine could be methylated by formalin and formic acid if acidity were avoided.

In an application of the above argument homoveratrylamine was heated with formalin while the pH was kept close to 7 by addition of formic acid. Toward the end of the operation enough acid was admitted to give a pH of 5. Despite these precautions, the yield of N,N-dimethylhomoveratrylamine was only 44%. In addition there was obtained a 14% yield of 2-methyl-6,7-dimethoxytetrahydroisoquinoline and a small amount of a substance apparently isomeric with the latter.

It appears consequently that the Pictet–Spengler cyclization is considerably more facile than has previously been supposed and that when the structural peculiarities of a phenethylamine are favorable for this cyclization the Clarke–Eschweiler reaction cannot be manipulated to avoid the cyclization completely. To some extent this can be rationalized if one assumes that both reactions proceed through an alkylolamine intermediate which must become cationic for either reaction to proceed. Such an assumption is generally made for the Pictet–Spengler reaction and is not unreasonable for the Wallach reaction.

$$\mathrm{RNHCH_2OH} \xrightarrow[\mathrm{H_3O^+}]{}$$

$$\underset{\mathrm{I}}{\mathrm{RN\overset{+}{H}_2CH_2OH}} \rightleftarrows \underset{\mathrm{II}}{\mathrm{R\overset{+}{N}H{=}CH_2 \longleftrightarrow RNH{-}\overset{+}{C}H_2}}$$

Two obvious cationic forms, I and the resonance hybrid II, can be written and the equilibrium between them might well be pH dependent. Thus, in the present instances, the essentially neutral medium may have minimized the tendency of I to pass into II without preventing it completely.

Experimental

Ten cc. of formalin was added to 9.1 g. (50 mmoles) of homoveratrylamine in a 3-necked conical flask set in a steam-bath and equipped with a stirrer, thermometer and dropping funnel. After the admission of 2.4 cc. of 90% formic acid the stirrer was started and the flask was heated to about 87°. The evolution of gas commenced at about 50°. Further quantities of 90% formic acid were added to maintain a pH of about 7. After three hours a total of 5 cc. of formic acid had been added. Five cc. more formalin was then run in and the reaction continued, 3 cc. more formic acid being added in the next 1.5 hour. The pH was then 5 and was unaltered by 1.5 hours further heating. The solution was evaporated *in vacuo*, 7 cc. of concentrated hydrochloric acid was added (change of color from brown to green) and the mixture was again taken down *in vacuo*. The residue was dissolved in absolute ethanol, ethyl acetate was added and the solution was seeded with N,N-dimethylhomoveratrylamine hydrochloride. The solid obtained, however, was not this expected salt since it melted at 204–208°. On recrystallization the melting point rose to 213–215° and was not depressed by admixture of authentic 2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride. The weight of this pure fraction was 1.7 g. (7 mmoles).

The mother liquors were evaporated and the bases were liberated and distilled *in vacuo* (below 1 mm.). The more volatile material (boiling below 120°) afforded, after crystallization of the hydrochlorides, 22 mmoles of pure N,N-dimethylhomoveratrylamine hydrochloride. The undistilled bases (*ca.* 3 g.) were dissolved in hot hexane, a small amount of tar was removed, and attempts were made to crystallize the bases. As these were unsuccessful the material was reconverted to the hydrochlorides and 0.8 g. of a solid melting at 224–225° was obtained. Recrystallization from ethanol–ether mixture raised this melting point to 229–230° dec. Higher decomposition points could be obtained if the bath was heated rapidly (242°, 239°), but all these were decomposition points and none were so high as those reported for 6,7-dimethoxytetrahydroisoquinoline hydrochloride (253°, 262°)[9] which is said to melt without obvious decomposition.

Anal. Calcd. for $C_{12}H_{18}ClNO_2$: C, 59.1; H, 7.4. Found: C, 59.3; H, 7.4.

The composition is thus consistent with this substance being a dimethoxy-N-methyltetrahydroisoquinoline hydrochloride. Permanganate oxidation yielded an acid whose identity is as yet uncertain.

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[5] *Cf.* "Organic Reactions," Edited by Roger Adams, Vol. VI, John Wiley and Sons, Inc., New York, N. Y., 1951, p. 151.

[6] J. S. Buck, This Journal, **56**, 1769 (1934).

[7] C. Schöpf and H. Bayerle, *Ann.*, **513**, 190 (1934). Actually a considerable gap exists between the conditions of Schöpf (dilute neutral solution and room temperature) and those customary in the synthetic reaction (high acidity, relatively high concentration and steam-bath temperatures). So far as the cyclization is concerned, concentration should affect only the formation of the methylolamine intermediate.

[8] E. Staple and E. C. Wagner, *J. Org. Chem.*, **14**, 559 (1949).

[9] J. S. Buck, This Journal, **56**, 1769 (1934); R. Forsyth, C. I. Kelly and F. L. Pyman, *J. Chem. Soc.*, **127**, 1659 (1925).

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Identification Derivatives of 2-Aminofluorene and of 2-Aminofluorenone

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The following new derivatives of 2-aminofluorene have been prepared and characterized.

2-Fluorenyl Phenyl Thiourea.—A solution of 1 g. of 2-aminofluorene in 50 ml. of ethanol and 2 g. of phenyl isothiocyanate was heated to boiling. Upon cooling, white needles separated. After recrystallization from ethanol, the m.p. was 179–180° uncor.

Calcd.[2] for $C_{20}H_{16}SN_2$: N, 8.86. Found: N, 9.40.

N-(2-Fluorenyl)-3-nitrophthalimide.—A mixture of 1 g. of 2-aminofluorene, 1.5 g. of 3-nitrophthalic anhydride and 15 ml. of dimethylaniline (solvent) was refluxed for 2 hours at the b.p. While still hot, 100 ml. of 95% ethanol was added. Shiny, golden flakes amounting to 0.4 g. when recrystallized from benzene of m.p. of 255.6° uncor. were obtained.

Calcd. for $C_{21}H_{12}O_4N_2$: N, 7.86. Found: N, 7.94.

New Derivatives of 2-Amino-9-fluorenone. N-2-(9-Oxo)-fluorenyl-4-nitrobenzamide.—A mixture of 0.5 g. of 2-aminofluorenone, 0.5 g. of p-nitrobenzoyl chloride and 10 ml. of pyridine was brought to the boiling point. While still hot, the mixture was filtered, diluted with 50 ml. of

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[1] Abstracted from the M.S. thesis of W. W. Muelder.

[2] Analyses were performed by Dr. Carl Tiedcke of Teaneck, N. J.

ethanol and allowed to cool. Orange-yellow crystals were obtained which after recrystallization from ethanol did not melt under 340°.

Calcd. for $C_{20}H_{12}N_2O_4$: N, 8.14. Found: N, 8.16.

N-2-(9-Oxo)-fluorenylbenzamide.—A mixture of 0.5 g. of 2-aminofluorenone, 0.36 g. of benzoyl chloride and 10 ml. of pyridine was heated to boiling. While still hot, the solution was filtered, diluted with 50 ml. of ethanol and allowed to cool. Vermillion crystals were obtained which after recrystallization from ethanol melted at 242° uncor.

Calcd. for $C_{20}H_{13}NO_2$: N, 4.68. Found: N, 4.89.

2-(9-Oxo)-fluorenylphenylurea.—A mixture of 0.5 g. of 2-aminofluorenone, 0.6 g. of phenyl isocyanate and 50 ml. of absolute ethanol was heated slowly to boiling. Crystals formed immediately but the mixture was allowed to cool. After three recrystallizations from ethanol the bright orange needles melted at 268°, resolidified and remelted at 323–324° (all uncor.).

Calcd. for $C_{20}H_{14}N_2O_2$: N, 8.92. Found: N, 9.18.

2-(9-Oxo)-fluorenylphenylthiourea.—A mixture of 0.5 g. of 2-aminofluorenone, 0.4 g. of phenyl isothiocyanate and 50 ml. of ethanol was brought to the boiling point and then allowed to cool. After 3 recrystallizations from dilute ethanol, the yellow-orange crystals melted at 185°.

Calcd. for $C_{20}H_{14}N_2SO$: N, 8.49. Found: N, 8.40.

2-Aminofluorenone oxime, prepared in the usual way and recrystallized from dilute ethanol, separated as orange yellow needles melting at 216–217° uncor.

Calcd. for $C_{13}H_{10}N_2O$: N, 13.33. Found: N, 13.29.

2-Aminofluorenone semicarbazone, prepared in the usual way, did not melt under 325° when repeatedly crystallized from ethanol.

Calcd. for $C_{14}H_{12}ON_4$: N, 22.22. Found: N, 21.69.

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The Role of Water Vapor on the Stoichiometric Reactions of Solid Proteins with Polar Gases[1]

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In a very interesting paper,[3] Czarnetsky and Schmidt (henceforth C. and S.) reported the reversible addition of acidic and basic gases such as CO_2, H_2S, NH_3 to proteins in the solid state. Such results, if verified, would have great importance both as an analytical tool in establishing the number and type of functional groups in the protein molecule and as a structural tool in clarifying the configuration, thermodynamic properties and spatial availability of these groups in the protein molecule.

C. and S. reported that on measuring the sorption isotherms of the above gases on solid proteins, isobaric regions were observed (*i.e.*, regions in which the proteins adsorbed the gases at constant partial pressures). Such isobaric regions of adsorption can be interpreted by means of the phase rule as corresponding to the formation of new, pure, solid phases and the amount of gas adsorbed would then correspond quantitatively to the amount of the new phase, in short, a stoichiometric, gas phase titration.

These results are, however, in contradiction to the results obtained in our own[4,5] and other laboratories.[6–8] The discrepancies may be summarized as follows: 1. The vapor pressures of the isobaric regions reported by C. and S. are in general different from those found in our own[4,5] and other laboratories.[6,7] 2. Only one pressure flat is found by C. and S. and this is ascribed to chemical combinations of some 5 or more different components of the protein (*e.g.*, arginine, lysine, etc.). One would expect each hydrochloride to be formed at a different equilibrium pressure. 3. An anomalous type of hysteresis is found by C. and S. in the adsorption of HCl on gelatin. 4. C. and S. find completely reversible sorption of HCl on gelatin. This is again in direct conflict with the previous findings of ourselves and others.[4–7]

Because of the above inconsistencies it was decided to repeat the experiments with gelatin and dry HCl gas. For these purposes a conventional apparatus similar to the one used by C. and S. was employed with certain modifications which have been described elsewhere.[4] The results which were obtained were consistent with those we had obtained previously with other proteins. There was no region of stable, reproducible[9] isobaric sorption. The desorption curve lay considerably above the sorption curve and was typical of hysteresis found with other proteins. Finally the desorption did not continue to zero HCl pressure but was completed (at 10^{-6} mm. pressure) at an amount of HCl sorbed, quantitatively equal to the sum of the arginine, histidine and lysine residues present in the gelatin sample.

These experiments, however, differed in one important respect from those reported by C. and S. The latter reported that in order to obtain reproducible results it was necessary to have catalytic traces of water vapor present. To achieve this they attached a side arm to their sorption buret containing $Na_2SO_4 \cdot 10H_2O$–Na_2SO_4 mixtures. At constant temperature this would provide a constant vapor pressure of water in the system. While they gave none of the specific details of the procedure followed they did mention having to then correct their pressure readings by a constant amount (*ca.* 1 mm.),[10] presumably that due to the water vapor thus introduced. They did not say whether or not the side arm with the $Na_2SO_4 \cdot 10H_2O$ was left open to the system during the sorption runs, although this seems implied by some of their subsequent discussion.

To check on these presumed effects of water

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[2] Graduate Research Fellows on United States Public Health Service Grant (G-3541).

[3] E. Czarnetsky and D. Schmidt, *J. Biol. Chem.*, **105**, 301 (1934).

[4] S. W. Benson and J. Seehof, THIS JOURNAL, **73**, 5053 (1951).

[5] Benson and Seehof, *ibid.*, **75**, 2427 (1953).

[6] W. D. Bancroft and C. J. Barnett, *J. Phys. Chem.*, **34**, 449 (1930).

[7] B. Belden, *ibid.*, **35**, 2164 (1931).

[8] R. Green, *Trans. Proc. Roy. Soc. New Zealand*, **78**, 291 (1950).

[9] Transient isobaric regions have been found which disappear if sufficient time is allowed to elapse to reach equilibrium.[4,5]

[10] This vapor pressure correction is rather mysterious since it does not correspond to any vapor pressure of the decahydrate easily accessible under laboratory conditions.