Jointly, these results show that sophorose, far from being a rare sugar, is the commonest glucose-containing disaccharide associated with the flavonoids. Gentiobiose is the only other glucose disaccharide yet found as a component of flavonoids, and pigments with this sugar are only known to occur in two plants, namely *Primula sinensis*[7] and *Tritonia*[11].

It is interesting to compare this finding of glucosyl (1 → 2)-β-glucose in flavonoid pigments with the situation in plant polysaccharides, in which β 1 → 4 and β 1 → 6 links are the most common; and with the situation in phenolic glycosides, formed when phenols are fed to plants, in which the only linkage found is β 1 → 4[19,20]. The enzyme system catalysing the synthesis of β 1 → 2 links thus appears to be rather localized in its distribution in the plant and to display a considerable degree of specificity. This is borne out by the fact that among all the plants so far found to contain sophorose, no example has been found where anthocyanins and flavones co-occurring are both linked to this sugar. This, in effect, means that whenever a second glucose is transferred to a flavonoid monoglucoside, the system catalysing the transfer is specific for anthocyanidin or flavonol. *Primula sinensis*, in which related anthocyanidin and flavonol 3-gentiobiosides and 3-triglucosides have been found[7], is the only known exception to this rule[21].

Zusammenfassung. Der seltene Zucker Sophorose konnte mit der Disaccharid-Einheit von Anthozyanen identifiziert werden, die in Arten von *Brassica, Papaver, Phaseolus, Raphanus, Tropaeolum* und *Watsonia* vorkommen, und von Flavonen, die in *Helleborus, Petunia, Pisum, Rosa* und *Solanum* vorhanden sind. Es scheint das am häufigsten auftretende, glucosehaltige Disaccharid mit diesen Farbstoffen übereinzustimmen.

J. B. HARBORNE

John Innes Institute, Bayfordbury (Hertford, Herts, England), August 21, 1962.

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The Effect of some Surface Active Drugs on the Assimilation of Ammonium Ions by a Strain of *Pseudomonas aeruginosa*

There is general agreement that surface active agents are adsorbed on the cell surface of bacteria and yeast. At certain concentrations this adsorption results in a leakage from the cell of potassium[1,2], amino acids[3], purines and pyrimidines[4] and undoubtedly other cell constituents. At the same time the oxygen uptake and CO_2 production may be increased[5,6] and certain substrates not oxidized by the normal cell may be oxidized presumably because the substrate has access to the enzyme once a barrier has been removed by the drug[1]. Some enzymes are inhibited[7–10].

In the following, it will be shown that a strain of *Pseudomonas aeruginosa* assimilates ammonium ions in the presence of an oxidizable substrate and sodium and potassium ions. In the absence of potassium ions very little assimilation occurs. Assimilation can be restored however by the addition of a proper concentration of either polymyxin B or benzalkonium chloride. The anionic detergent sodium alkylbenzene sulfonate is relatively ineffective although it and the cationic compounds may increase the oxidation rate of the substrate.

A strain of *Pseudomonas aeruginosa* kept in this laboratory for fifteen years was grown for 24 h at 34° on Bacto Nutrient Broth. The cells were then centrifuged down, washed twice with water and finally suspended in 0.05 *M* Na phosphate buffer to a standard optical density. Despite this, there was considerable variation from experiment to experiment. Since the number of molecules

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The effect of 10 μg/ml polymyxin B, 5 μg/ml benzalkonium chloride, 80 μg/ml sodium alkylbenzene sulfonate, and 0.025 *M* potassium phosphate pH 7.7 on the oxidation of 1.0 mg sodium succinate ($6H_2O$) on the assimilation of ammonium ions. The cells were suspended in 0.05 *M* sodium phosphate buffer pH 7.7 except when potassium was present when 0.025 *M* sodium buffer was used. The concentrations of the drugs are optimal or very near it. 26 μg of NH_3-N was added as NH_4Cl

Additions	Incubation time min	O_2 uptake μl	μg NH_3-N assimilated	μg NH_3-N × 100 / μl O_2	Mean and S.D. of ratio for 6–8 experiments
None	80	113	4	3.5	2.5 ± 1.8
Benzalkonium chloride	80	107	9	8.4	8.6 ± 3.4
Polymyxin B	80	129	9	7.0	7.3 ± 4.1
Sodium alkyl benzene sulfonate	80	137	6	4.4	4.3 ± 1.3
KH_2PO_4	40	85	8	9.4	10.5 ± 1.4

of drug per cell appears to be critical, small variations in cell number and drug concentration may produce relatively large effects. The effects of the drugs on the assimilation, however, were consistent. Oxygen uptake measurements were made in Warburg vessels containing 2.0 ml fluid volume. Ammonia determinations were made on the supernate of cells which had been centrifuged down after the addition of 0.2 ml 20% trichloroacetic acid. After suitable dilution, Nessler's reagent was added and the color read at 420 mμ.

The Table shows that the addition of potassium is necessary for effective ammonia assimilation as shown by the ratio of ammonium ions assimilated to oxygen taken up. This ratio is not affected by the time of the incubation until the substrate concentration becomes limiting. The potassium requirement may be the result of potassium loss which occurs when the cells are washed in water. In 40 ml of wash water there were on the average 5.7 m*M* of potassium. The estimations were made with the flame photometer. It also shows that polymyxin B and benzalkonium chloride can restore ammonia assimilation to a significant degree. That the anionic drug is less effective suggests that the elimination of a barrier to ammonium ions is not the only mechanism of increased assimilation but that the cationic drugs may be substituting for potassium in this reaction. When the drugs are added in this or lower concentrations to the cells in the presence of potassium, they are without effect.

Riassunto. Cellule di *Pseudomonas aeruginosa* perdono potassio durante lavaggio in acqua e contemporaneamente esibiscono bassi livelli di utilizzazione di ioni ammonio in presenza di substrato ossidabile. Un rilevante aumento di utilizzazione di ammonio è indotto dall'aggiunta di potassio o detergenti cationici (polimixina B, cloruro di benzalkonio), ma non dall'aggiunta di un detergente anionico.

F. Bernheim

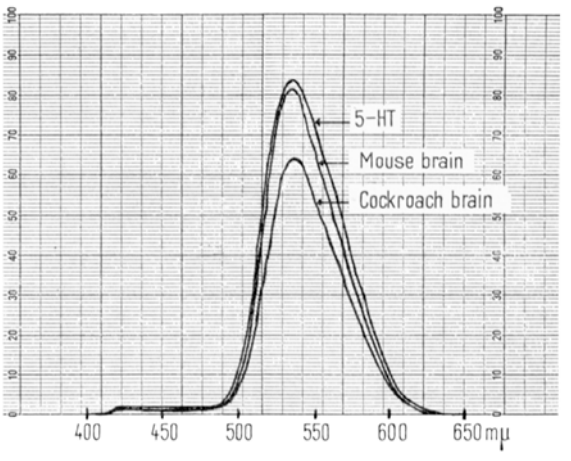
Department of Physiology and Pharmacology, Duke University Medical Center, Durham (North Carolina, U.S.A.), July 2, 1962.

Synthesis of 5-Hydroxytryptamine in the American Cockroach

In insects venom glands 5-hydroxytryptamine (5-HT) has been identified with some degree of certainty (Jaques and Schachter[1], Erspamer[2], Bhoola et al.[3]). The amounts of the compound are much in excess of the values reported by Welsh and Moorhead[4] for ground up bodies of several insect species. Davey[5] indicated that a gland (utriculi majores) of the reproductive system of the male American cockroach contained an *o*-dihydroxyindolealkylamine. The properties of the substance would appear to be similar to those of a compound found in pericardial organs of Crustacea (Carlisle[6]), and subsequently stated to be 5,6-dihydroxytryptamine (5.6-diHT) by Carlisle and Knowles[7]. However, Gersch et al.[8] believe that nervous tissue of the cockroach contains 5-HT. Colhoun and Blaschko (in manuscript) have shown that 5,6-diHT was almost as pharmacologically active as 5-HT when tested upon the rat fundus muscle preparation. Unpublished observations have shown that both compounds at 10^{-8} to $10^{-9}M$ increased the rate of contraction of fore and hind gut, movement of malpighian tubules and rate of heart beat of the American cockroach. Bromolysergic acid diethylamide was an efective blocking agent. Although of pharmacological interest these observations are of little importance in the physiology of the cockroach until the distribution and identity of endogenous indole compounds is established.

A large number of tissues of the cockroach were subjected to extraction procedures for differential bioassay, ultraviolet absorption and spectrophotofluorometer techniques for the identification of indole compounds. Briefly, the results show the presence of a substance with the characteristics of 5-HT in brain, corpora cardiaca glands and ventral nerve cord. On a tissue weight basis the highest amount was found in glands, then brain and ventral nerve cord. Because of loss of active substance during the overall extraction procedure for chromatography and subsequent bioassay, the actual amount of 5-HT in tissues has not been determined. The amounts are extremely low in comparison with the titre of acetylcholine in brain and ventral nerve cord. Indeed the small quantity of 5-HT suggests localized distribution in nervous tissue.

Results with other tissues such as gut, malpighian tubules and utriculi majores have been inconclusive. In comparison with data for neural tissue it would seem possible that these tissues contain a substance not identified as 5-HT, or, equally important, 5,6-diHT.



Fluorescence spectra in 3*N* HCl of synthesized 5-HT from mouse and cockroach brain. Excitation monochromator set at 300–305 mμ.

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