CHANGES OCCURRING IN SOLUTIONS FOR INJECTION CONTAINING PROCAINE HYDROCHLORIDE AND DEXTROSE

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The work here described originated in the observation of apparent anomalies in the optical rotations of solutions containing procaine hydrochloride and dextrose. Such solutions have, when freshly prepared, the expected optical rotation calculated from the dextrose content. On standing at room temperature, however, the rotation slowly changes until, after a period of about 3 weeks, it finally becomes constant at a value opposite in sign to that of freshly-prepared solution.

TABLE I

THE CHANGES IN OPTICAL ROTATION OF AN AQUEOUS SOLUTION CONTAINING PROCAINE HYDROCHLORIDE 5 PER CENT. AND DEXTROSE (ANHYDROUS) 5 PER CENT. STORED AT ROOM TEMPERATURE

Time (days)	Optical Rotation (angular degrees for 2 dm. column)
0	+2·50
1	+2·30
2	+1·90
5	+0·55
7	0·00
10	−0·60
15	−1·30
20	−1·65 (constant)

Analogous changes in the optical rotations of solutions containing procaine hydrochloride with other sugars have also been observed; i.e., with the aldoses, D(−)arabinose, L(+)arabinose, D(+)galactose, lactose, maltose and D(+)xylose. No changes in rotation were observed in solutions containing procaine hydrochloride with the ketoses D(−)fructose and L(−)sorbose, the hexitols mannitol and dulcitol, or the non-reducing sucrose and raffinose. With different aldoses the changes occur at considerably different rates; with arabinose equilibrium is reached in 2 or 3 days, while with maltose about 15 days are required at 25°C. Figure 1 shows the changes in optical rotations of solutions of procaine hydrochloride with different sugars. All the solutions were 0·25 molar with respect to both procaine hydrochloride and sugar. In order to avoid complications arising from mutarotation, the sugar solutions were boiled and cooled before the procaine hydrochloride was added. They were stored at 25°C. Figure I shows the optical rotation of a 2 dm. column of the solution.

Similar changes occur in solutions containing dextrose with certain other local anæsthetics. Those reacting in this way include amylsine,

panthesine and butacaine sulphate, all of which are, like procaine, primary amines. There is no similar interaction between dextrose and either amethocaine hydrochloride or amylocaine hydrochloride, neither of which is a primary amine.

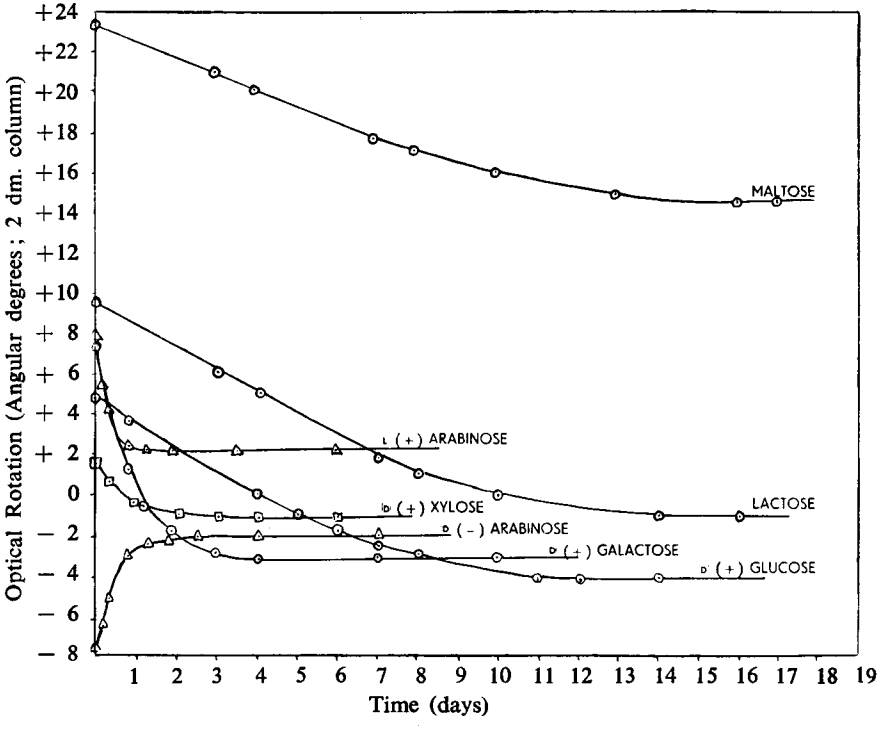


FIG. 1. Changes in optical rotation of solutions containing procaine hydrochloride with various sugars.

Table II shows the changes in the optical rotation of solutions of dextrose with some different local anæsthetics. All solutions were 0·1 molar with respect to both dextrose and local anæsthetic and were stored at 25°C. The rotations shown are those observed for 2 dm. columns of the solution.

TABLE II

	Original Value	Final Value
Amylsine	+1·90°	−0·55°
Butacaine sulphate	+1·90°	−0·24°
Panthesine	+1·90°	−0·30°

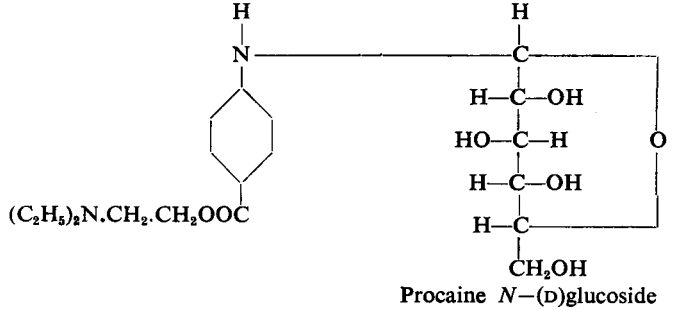
The reaction responsible for the changes in optical rotation appears, therefore, to take place only between those sugars which behave as aldoses and those local anæsthetics which are primary amines. Changes of this

sort occurring in local anæsthetic solutions do not appear to have been previously reported and therefore were considered worthy of investigation. The reaction between procaine hydrochloride and dextrose was selected for further study.

Primary aromatic amines react readily with aldoses in either aqueous or alcoholic solution, with the formation of *N*-glycosides[1,2,3]. The reaction is a condensation involving the amino group of the amine and the reducing group of the sugar. The changes occurring in solutions of procaine hydrochloride and dextrose could most readily be explained in terms of this reaction, and it has been found that a condensation of this sort does, in fact, take place. A crystalline compound has been prepared by the interaction, in aqueous solution, of procaine hydrochloride and dextrose. Its properties indicate that it is procaine-*N*-(D)glucoside, having the structure shown below. As this compound does not appear to have been described in the literature, some of its properties have been investigated and are reported.

The compound consists of small colourless crystals, m.pt. 140° to 141°C. Its elementary analysis agrees with that calculated for procaine *N*-(D)glucoside hydrochloride. It is readily soluble in water. Aqueous solutions are lævorotatory and show mutarotation, the compounds having $[\alpha]_D^{22.5°C}$ (equilibrium value) = −74·0°. Mutarotation is commonly shown by *N*-glycosides and may be due to the establishment of an equilibrium between the α and β isomers and the Schiff base.

The addition of alkali to solutions of the hydrochloride of the glucoside does not cause precipitation of the base, nor can the base be extracted by chloroform from alkaline solutions.



Procaine *N*—(D)glucoside

Aqueous solutions of the glucoside do not reduce Fehling's solution except on prolonged boiling. Nor do they give more than the faintest diazo reaction provided that the test is conducted so as to minimise contact with acid. The glucoside is readily hydrolysed in acid solution and the depth of colour produced is a function of the time the solution is allowed to remain acid. This compound is thus more stable than many others of the same class, which, because of the extreme ease with which they hydrolyse, readily give both reducing and diazo reactions. From these facts it is apparent that the procaine and the sugar are linked through the amino group of the one and the reducing group of the other.

The hydrolysis of procaine *N*-(D)glucoside in hydrochloric acid has been followed polarimetrically, and its course has been found to approximate to that of a first order reaction.

The dissociation constant of the glucoside has been calculated from the titration curve of the hydrochloride with sodium hydroxide. The glucoside (pK_b = 5·06) is a slightly stronger base than procaine (pK_b = 5·2). This conforms to the general rule that secondary amines are stronger bases than the corresponding primary amines and thus lends additional support to the proposed structure for the condensation compound.

EXPERIMENTAL

Preparation of Procaine N-(D)*Glucoside Hydrochloride.* 27·28 g. (1 mol.) of procaine hydrochloride and 18·02 g. (1 mol.) of anhydrous dextrose were dissolved in 80 ml. of water and heated on a boiling water-bath for 3 hours. The solution was then evaporated almost to dryness under reduced pressure, the residue dissolved in boiling 95 per cent. ethanol, filtered and allowed to crystallise. The crude product (40 g.) had m.pt. 136° to 138°C. It was twice recrystallised from 95 per cent. ethanol and finally dried over sulphuric acid. The product (20 g.) had m.pt. 140° to 141°C.; this was unaltered by further recrystallisations. Found, C, 50·1; H, 7·3; N, 6·96; Cl, 7·84; $C_{19}H_{30}O_7N_2,HCl,H_2O$ requires C, 50·4; H, 7·34; N, 6·2; Cl, 7·83 per cent.

On standing *in vacuo* over phosphorus pentoxide, this compound slowly loses its water of crystallisation, yielding a product of m.pt. 145° to 146°C. Found, Cl, 8·16; $C_{19}H_{30}O_7N_2,HCl$ requires Cl, 8·15 per cent The results reported in this paper refer to the monohydrate.

Optical Activity of Procaine N-(D)*Glucoside Solutions.* Table III shows the mutarotation at 22·5°C. of a freshly prepared aqueous solution of procaine *N*-(D)glucoside hydrochloride.

TABLE III

Time (hours)	Observed rotation (2 dm)
0·12	−18·14°
0·25	−17·35°
0·50	−16·58°
1·0	−15·94°
2·0	−15·33°
2·5	−15·14°
3·0	−15·03°
4·0	−14·89°
5·0	−14·80°
10·0	−14·79° (constant)

$$[\alpha]_D^{22.5} \text{ (equilibrium value)} = \frac{-14{\cdot}79 \times 100}{2 \times 10} = -74{\cdot}0°$$

The Dissociation Constant of Procaine N-(D)*Glucoside.* The hydrochloride of the glucoside was titrated with sodium hydroxide, each of the readings shown in Table IV being obtained in the following way. 0·2264 g. of procaine *N*-(D)glucoside hydrochloride was dissolved in water, 0·05 N sodium hydroxide solution (carbonate free) was added in the

proportions shown, and the volume was adjusted to 50 ml. The *p*H of each solution was measured electrometrically (glass electrode).

TABLE IV

Sodium hydroxide equivalents added	*p*H	*p*OH	(OH)	Dissociation Constant (K)	pK_b
0·0	6·30	—	—	—	—
0·1	8·10	6·04	$9·12 \times 10^{-7}$	$8·21 \times 10^{-6}$	5·086
0·2	8·49	5·65	$2·24 \times 10^{-6}$	$8·96 \times 10^{-6}$	5·048
0·3	8·67	5·47	$3·39 \times 10^{-6}$	$7·91 \times 10^{-6}$	5·100
0·4	8·89	5·25	$5·62 \times 10^{-6}$	$8·43 \times 10^{-6}$	5·074
0·5	9·07	5·07	$8·51 \times 10^{-6}$	$8·51 \times 10^{-6}$	*5·070*
0·6	9·26	4·88	$1·32 \times 10^{-5}$	$8·80 \times 10^{-6}$	5·055
0·7	9·42	4·72	$1·91 \times 10^{-5}$	$8·20 \times 10^{-6}$	5·086
0·8	9·67	4·44	$3·63 \times 10^{-5}$	$9·07 \times 10^{-6}$	5·042
0·9	10·02	4·12	$7·59 \times 10^{-5}$	$8·43 \times 10^{-6}$	5·074
1·0	10·56	—	—	—	—

The value at half neutralisation and also the mean value give

$$K = 8·51 \times 10^{-6} \text{ and } pK_b = 5·070.$$

The values for K were calculated from the relationship

$$K = \frac{(OH) \times (\text{Base Ion})}{\text{Undissociated Base.}}$$

It was assumed that the concentration of base was equal to the number of equivalents originally present (1) less the number of equivalents of sodium hydroxide added, and that the concentration of undissociated base was equal to the number of equivalents of sodium hydroxide added.

The Hydrolysis of Procaine N-(D)*Glucoside.* Table V shows the course of the hydrolysis of the glucoside in 0·2 N hydrochloric acid at 22·5°C. The velocity constant was calculated from the relationship.

$$K = \frac{1}{t} \log \frac{(a)}{(a - x)}$$

The course of the hydrolysis approximates to that of a first order reaction.

TABLE V

THE HYDROLYSIS OF A 5·00 PER CENT. SOLUTION OF PROCAINE *N*-(D)GLUCOSIDE HYDROCHLORIDE IN 0·2N HYDROCHLORIC ACID AT 22·5°C.

Time minutes	Observed rotation (2 dm)	Hydrolysis per cent.	K (min^{-1})
0	−7·40°	—	—
10	−5·00°	25·26	0·0126
20	−3·38°	42·32	0·0120
30	−2·20°	54·74	0·0115
45	−1·11°	66·21	0·0105
60	−0·29°	74·84	0·0100
120	+1·16°	90·11	0·0084
—	+2·10°	100·00	—

Solutions in which hydroylsis is complete have optical rotations identical with those calculated for the dextrose produced from procaine N-(D)glucoside. Determination of the procaine in completely hydrolysed solutions, by extraction with chloroform followed by acidimetric titration, shows that procaine is produced in the proportion calculated for procaine *N*-(D)glucoside.

DISCUSSION

Evidence has been presented indicating that in solutions containing procaine hydrochloride and dextrose, condensation can occur with the formation of procaine *N*-(D)glucoside, and this compound has been isolated and characterised. That similar reactions can take place between certain other local anæsthetics and certain other sugars, is indicated by changes in the optical activity of their solutions. As it seems reasonable to suppose that these changes are completely analogous to those occurring between procaine hydrochloride and dextrose, only the latter reaction has been investigated in detail. Further work is, however, being carried out on *N*-glycosides.

The formation of *N*-glycosides in local anæsthetic solutions does not seem to have been previously described, but the general reaction between primary amines and aldose sugars is well known[4,5,6,7]. Many *N*-glycosides have been isolated in the crystalline state, but in solution they are believed to exist in equilibrium with the corresponding Schiff base.

The occurrence of these condensation compounds in local anæsthetic solutions has analytical significance in that the determination of the sugar or the local anæsthetic itself must be preceded by hydrolysis of the glycoside. The pharmacological implications have not yet been investigated. It may be inferred from the continued successful use of such solutions that the action of the glucoside closely resembles that of procaine itself. Those *N*-glycosides which have been investigated pharmacologically have been found to have actions closely approximating to those of their aglycones[8,9,10].

SUMMARY

1. In solutions containing procaine hydrochloride and dextrose, condensation occurs with the formation of procaine *N*-(D)glucoside. Analogous condensations occur between other local anæsthetics which are primary amines, and other aldoses.

2. Procaine *N*-(D)glucoside, which has not been previously described, has been isolated and characterised.

3. Some implications of this reaction have been indicated.

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DISCUSSION

The paper was presented by the Author.

The CHAIRMAN said he understood that the pharmacological action of the glycoside had not been investigated but he saw no reason why it should differ from that of procaine hydrochloride.

DR. F. HARTLEY (London) said that it would be interesting to know whether buffering nearer to neutrality would affect the rate of glycoside formation.

DR. G. FOSTER (Dartford) said that the many glycosides of medicinal importance were known in which the genin exhibited pharmacological properties different from those of the glycoside itself, and it would be useful to know whether similar differences in properties existed between procaine hydrochloride and its glucoside.

MR. H. S. GRAINGER (Westminster) asked for further information as to the purpose for which the preparation referred to had been used.

MR. CANNELL, in reply, said that neither the rate of reaction nor the equilibria had been determined over a range of *p*H. If the *p*H were lowered the glycoside was hydrolysed and if it were raised to anything like alkalinity, the procaine itself was hydrolysed. The original observations were made on a routine batch but he could give no further information as to its purpose.