

THE ASSOCIATION OF GLUCOSE WITH *N*-4-NITROPHENYL- β -D-GLUCOSYLAMINE*

By H. H. HATT†

An addition compound formed between an organic compound and one of either glucose or fructose offers the possibility of separating these two sugars and of recovering the organic compound subsequently by liquid-liquid extraction. In this connection the addition compound between *N*-4-nitrophenyl- β -D-glucosylamine (I) and glucose, which Weygand, Perkow, and Kuhner¹ reported, has been examined. Tests by paper chromatography indicated that (I) would be more readily separable from glucose and fructose by liquid-liquid extraction than would, for example, urea which also complexes with glucose.²

Weygand, Perkow, and Kuhner assigned their compound the molecular formula: $C_{12}H_{16}N_2O_7 \cdot C_6H_{12}O_6 \cdot 4H_2O$ (II) and a melting point of 112°. However, the only analytical evidence given in support of this composition concerned the supposed anhydrous material and, as can be seen below, agrees less well with this postulated equimolecular composition than with one much poorer in glucose:

Found:	C, 45.07;	H, 5.90;	N, 7.4%
Calc. for $C_{12}H_{16}N_2O_7 \cdot C_6H_{12}O_6$:	C, 45.00;	H, 5.87;	N, 5.8%
Calc. for $(C_{12}H_{16}N_2O_7)_3 \cdot C_6H_{12}O_6 \cdot 2H_2O$:	C, 45.16;	H, 5.78;	N, 7.5%

When the work of Weygand, Perkow, and Kuhner was repeated it was noticed that, in the presence of dissolved glucose, *N*-4-nitrophenyl- β -D-glucosylamine loses its habit of crystallizing in well-formed long fine needles and instead separates as spherulites of ill-shaped microcrystals suggestive of contamination rather than of the formation of a well-defined addition compound. In fact, it has not proved possible either by following the directions of Weygand, Perkow, and Kuhner or by crystallizing glucose and (I) together under a variety of conditions to obtain their equimolecular compound. No addition compound of stoichiometric composition has been obtained. When (I) is crystallized in presence of glucose it entraps an amount of this sugar which varies with the conditions used, but which is always far less than the 37.5% required by (II) (dry basis) and is often only a few per cent. As noted, the analytical data of Weygand, Perkow, and Kuhner also give support to this finding. However, (I) shows some degree of preference for association with glucose for, under similar conditions, fructose is not detectably entrapped. Weygand, Perkow, and Kuhner found that the glucose and compound (I) in their addition compound were readily separable by paper chromatography and this has been confirmed for the complexes now prepared. The intensity of the glucose spot, after staining with silver nitrate and alcoholic alkali, can be used in a comparison method to estimate the amount of

* Manuscript received November 7, 1963.

† Sugar Research Laboratory, CSIRO, Biochemistry Department, University of Melbourne.

¹ Weygand, F., Perkow, W., and Kuhner, P., *Chem. Ber.*, 1951, **84**, 594.

² Hatt, H. H., and Triffett, A. C. K., *J. Chem. Soc.*, 1963, 2079.

glucose present in a crystalline complex to within 1 or 2%. By this means it was found that the amounts of glucose present in the solid products obtained by crystallizing equimolecular mixtures of (I) and glucose from aqueous ethanol under a variety of conditions, varied from 4 to 8% of glucose on a dry basis. The amounts of (I) present could be determined by a spectrophotometric method to within 1%. In aqueous solution (I) shows an absorption band at λ_{max} , 371 m μ ; ϵ_{max} , 16,200; and at the dilutions used for measurement this value is unaffected by the presence of an equimolecular amount of glucose. The amounts of (I) present in the products from its co-crystallization with glucose, mentioned above, were all 90% or more and thus supported the estimations of glucose.

Elementary analyses of preparations can give only less exact information concerning composition. Most of the complexes could be fitted to a general formula $(\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_7 \cdot \text{H}_2\text{O})_n \cdot \text{C}_6\text{H}_{12}\text{O}_6$ in which n was 4 or greater. Thus by following the directions of Weygand's group for the preparation of their addition compound, the crude product obtained after washing with alcohol and with ether contained 73% of (I) and 15–20% of glucose. This was dissolved in warm 95% ethanol (40 parts) and cooled to -2° . Crystallization commenced below room temperature (compare¹). The crystalline complex was analysed. Found: loss at 80° (*in vacuo*) 5.7%. Found (anhydrous): C, 46.8; H, 5.6; N, 8.5%. Calc. for $(\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_7 \cdot \text{H}_2\text{O})_4 \cdot \text{C}_6\text{H}_{12}\text{O}_6$: loss 5.1%; anhydrous: C, 47.0; H, 5.6; N, 8.1%. Calc. for $(\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_7 \cdot \text{H}_2\text{O})_5 \cdot \text{C}_6\text{H}_{12}\text{O}_6$: loss, 5.4; C, 47.1; H, 5.5; N, 8.3%. This impure (I) was readily freed of all contaminating glucose by a further crystallization from hot 95% methanol. The solvents used in all crystallizations contained 0.05% of ammonia ($d = 0.880$).

Weygand, Perkow, and Kuhner obtained their "addition compound" by using essentially the method of Berger and Lee³ for the preparation of arylglycosylamines. They interrupted the reaction after 3 days at a stage when it was still incomplete. Moreover the amount of water they used was barely sufficient to keep the glucose in solution and in some of the experiments made here under these conditions, part of the glucose crystallized during the three days as its hydrate. However, the method can be changed to give at once a nitrophenylglucosylamine contaminated with only 1–2% of glucose. Such a product crystallizes spontaneously from the reaction mixture of Weygand, Perkow, and Kuhner if the reaction time exceeds 10 days. If the amount of water and the amount of hydrochloric acid used are increased slightly then a usable method of preparation results.

Anhydrous glucose (4.5 g) was dissolved in warm water (6 ml) and the solution cooled. *p*-Nitroaniline (4.5 g) was dissolved in warm 97% ethanol (100 ml), 2*N* HCl (2 ml) was added, the solution cooled, and then mixed with that of glucose. After 3 days the crystalline product was collected and washed several times with alcohol and with ether. Yield, 5.3 g (70%) or, after 10 days, 6.3 g (84%). This product contained 98% of (I) and only a trace (1%) of glucose, but possessed the poor crystalline form induced by contamination with glucose. It melted at $140\text{--}175^\circ$, but mainly from 160° onwards and with loss of water from about 110° . After one recrystallization from 95% methanol it formed pale yellow needles, m.p. $182\text{--}187^\circ$ (with decomp.).

The spectroscopic measurements were made on a Beckman DK2 instrument. The upper layer of a 4 : 5 : 1 mixture of *n*-butanol–water–ethanol was used in descending paper chromatography.

³ Berger, L., and Lee, J., *J. Org. Chem.*, 1946, 11, 75, 84.