Table 2.

	1. Weight p.c.	2. Atomic quot.	3. Number of atoms and H_2O mol.	4. $Ca_3Al_2F_{12} \cdot 4\ H_2O$ weight p.c., calc.
Al	11.58	0.429	1.00	11.37
Ca	24.30	0.606	1.41	25.35
F	49.01	2.579	6.01	48.08
H_2O+	15.35	0.852	1.99	15.20
Total	100.24			100.00

From the values in column 3 we get the ideal formula: $Ca_3Al_2F_{12} \cdot 4\ H_2O$.

Already a glance at Dorfman's formula shows that it cannot possibly be correct, stating as it does that the percentage content of Ca and Al is about the same.

If we calculate the molecular weight from the figures in column 2, we get $M = 2 \cdot 233.5 = 467.0$.

By inserting Kvitka's cell dimensions, the specific weight and M we get:

$$Z = \frac{13.47 \cdot 8.46 \cdot 9.89 \cdot 2.720}{1.6604 \cdot 467.0} = 3.96$$

That is to say 4 formula units ($Ca_3Al_2F_{12} \cdot 4\ H_2O$) in the unit cell.

The present author has previously published a paper [2] in which amongst other things is described a "Ca-cryolite", produced by the wet method, with the formula $Ca_3Al_2F_{12} \cdot 3\ H_2O$, *i.e.* chemically closely allied to belyankite, with the same general properties (very readily fusible, easily dissolving in warm diluted hydrochloric acid). Bøgvad [3] has determined the specific weight for "Ca-cryolite" at 2.77 in good agreement with Dorfman's value 2.720.

1. Dorfman, M. D. *Doklady Akad. Nauk S.S.S.R.* **75** (1950) 851. Ref. *Chem. Abstr.* **45** (1951) 8943.
2. Nielsen, A. H. *Z. anorg. allg. Chem.* **232** (1937) 155. Ref. *Neues Jahrb. Mineral.* (1937) 595—6.
3. Bøgvad, *Z. anorg. allg. Chem.* **232** (1937) 156.

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Reaction of Organophosphorus Compounds with Dihydroxyphenyl Derivatives

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From both a practical and theoretical point of view we are interested in the reaction mechanism of organophosphorus compounds. We know that these agents inhibit cholinesterases and that this most probably explains their toxic effects. We also know that the inhibitory reaction is a phosphorylation at the so-called esteratic site of the enzyme molecule.

We have tried to protect *in vitro* cholinesterase preparations against inactivation by various organophosphorus compounds. About 120 various compounds have been tested as protectors. The most promising results were obtained with some local anesthetics. It could be demonstrated, for instance, that procaine at a concentration (5×10^{-5} M) which has no measurable effect on the serum cholinesterase activity protects this enzyme against inactivation by Tabun. In the case of acetylcholinesterases from various sources (erythrocytes, nucleus caudatus, cobra venom) the protective effect is less, but a definite protection has been demonstrated in all cases [1].

The protective action has further been demonstrated when inactivator and protector are simultaneously mixed with the enzyme; in these instances inactivator and protector were mixed together about one hour before mixing with the enzyme. In the procaine experiments, the protective effect was the same, as in those experiments when the enzyme was incubated with the protector prior to the inactivator. With some other compounds tested, however, it was shown that Tabun and DFP react

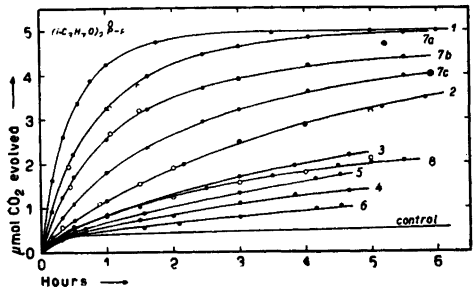

Fig. 1. Reaction of 5 µmol DFP with various compounds in 0.025 M $NaHCO_3$. pH 7.9. 25.0° C. Numbers in the following list refer to µmol, unless otherwise stated: 1) Pyrogallol 100; 2) Pyrocatechol 25 and DOPA 25; 3) Hydroquinone 100; 4) Resorcinol 100; 5) Phloroglucinol 100; 6) Phenol 100; 7a) Adrenaline 100; 7b) Adrenaline 50 and noradrenaline 50; 7c) Adrenaline 25; 8) Histamine 50 and cobra venom 19.2 mg.

with the "protector" to give a product less active as an inactivator of the enzyme. Pantocaine was the first compound tested which showed this effect.

The reaction between the "protector" and Tabun or DFP, however, was more pronounced with a series of di- and trihydroxyphenyl derivatives. For instance, it was shown that pyrocatechol reacts with DFP and Tabun. The inhibitory effect was decreased more the longer pyrocatechol was incubated with DFP or Tabun. When the reaction between DFP and pyrocatechol was tested in a bicarbonate solution in the Warburg apparatus, production of acid could be demonstrated. Pyrogallol gave a higher reaction rate with DFP. At that time a paper by Jandorf and co-workers [2] announced a similar observation. A great number of compounds have since been tested in this way in our laboratory, and some of the results are illustrated in Fig. 1.

Among the dihydroxyphenyl derivatives the most important ones are adrenaline and noradrenaline, which react with DFP in a bicarbonate solution with the evolution of CO_2. The reaction rate is the same for both adrenaline and noradrenaline. DOPA (3,4-dihydroxyphenyl-alanine) reacts with DFP at the same rate as pyrocatechol.

Like Jandorf and co-workers, we interpret this observation as due to phosphorylation of a phenolic group. With DFP, hydrofluoric acid is liberated and a diisopropylphosphate of the dihydroxyphenyl derivative is obtained. One molecule of DFP reacts with one molecule of the phenol. Even when the latter is 100 fold excess there is only this one to one relationship. The reaction is of second order or pseudo-first order.

Obviously one is immediately inclined to think of the reaction between DFP and cholinesterase as having a similar mechanism. If therefore a high concentration of cholinesterase is allowed to react with DFP the production of hydrofluoric acid should be detectable using the same technique. This was also the case when a relatively high concentration of cobra venom esterase reacted with DFP (Fig. 1).

The other organophosphorus compound which has been tested in detail in Sweden is Tabun. A comprehensive pharmacological study has been made by Holmstedt [3] and the physico-chemical properties of Tabun are reported by Larsson [4]. We have now demonstrated that Tabun also reacts

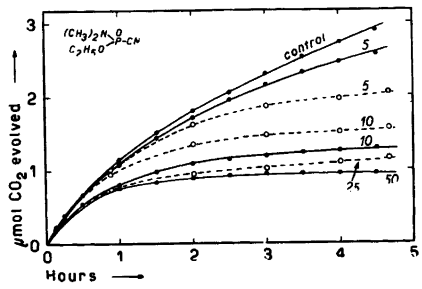

Fig 2. Reaction of Tabun with pyrogallol and adrenaline O in 0.025 M $NaHCO_3$. pH 7.9. 25.0° C. Numbers refer to µmol pyrogallol and adrenaline. Approximately 4 µmol Tabun.

with dihydroxyphenyl derivatives. The reaction with adrenaline is illustrated in Fig. 2. At pH 8 Tabun is hydrolysed yielding hydrocyanic acid and dimethylamido phosphoric acid [4]. The more adrenaline present the less acid is produced and we explain this by postulating a phosphorylation of adrenaline during the hydrolysis.

The inhibitory effects of the reaction products on cholinesterase have been tested. A concentration of DFP which is more than 1000 times higher than that required to give 50 % inhibition does not give any inhibition at all when the DFP has been incubated with adrenaline for 10 hours or more; the concentration of adrenaline was 20 times the concentration of DFP. Similar results were obtained with Tabun.

I am greatly indebted to Mrs Britta Tyrefors for technical assistance and for the skill and care which she has bestowed on the experiments.

1. Augustinsson, K.-B. *Acta Physiol. Scand.* In press.
2. Jandorf, B. J., Wagner-Jauregg, T., O' Neill, J. J., and Stolberg, M. A. *J. Am. Chem. Soc.* **74** (1952) 1521.
3. Holmstedt, B. *Acta Physiol. Shand.* **25** (1951) suppl. 90.
4. Larsson, L. *Acta Chem. Scand.* **6** (1952). In press.

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Diheterolevulosan III and IV

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The recent publication by Sattler *et al.*[1] prompts us to report the isolation of two new compounds which are probably isomerides of the previously known diheterolevulosans I and II. The substances were isolated from the unfermentable residue from hydrochloric acid treated fructose [2] by fractionating the mixture on a column of animal carbon, using the gradient elution technique [3] combined with fractional crystallisation. Both substances are non-reducing and difficult to hydrolyse, and on paper chromatograms they travel at about the same speed as diheterolevulosan II. The analytical data for one of the substances is consistent with its formulation as a diheterolevulosan ($C_{12}H_{20}O_{10}$). It has not been possible to analyse the second compound as yet owing to certain technical difficulties in the micro analytical department, but the periodic acid oxidation and the general properties of the substance indicate a diheterolevulosan type of structure.

Diheterolevulosan III, m.p. 279—281° ** (dec.), $[\alpha]_D^{20}$ −309° (water, c = 2), (Analysis: Found C, 44.5; H, 6.12. Calc. for $C_{12}H_{20}O_{10}$ C, 44.44; H, 6.18 %), consumes 4 moles of periodic acid and forms 2 moles of formic acid, and is thus probably an anomer of diheterolevulosan I. Acetate: M.p. 268—269° (sublimes), $[\alpha]_D^{20}$ − 199° (chloroform, c = 2). Analysis: C, 50.2; H, 5.67. Calc. for $C_{24}H_{32}O_{16}$ C, 50.0; H, 5.55 %.

"Diheterolevulosan IV", m.p. 240—242° (dec.), $[\alpha]_D^{20}$ −183° (water, c = 2), consumes 3 moles of periodic acid and forms 1 mole of formic acid. Acetate: M.p. 130—131°, $[\alpha]_D^{20}$ −159° (chloroform, c = 2).

A more detailed report will be published shortly in this journal.

Added in proof (29.7.52): Since this note was submitted for publication Wolfrom, Hilton and Binkley (*J. Am. Chem. Soc.* **74** (1952) 2867) have described a new di-D-fructose dianhydride "diheterolevulosan III" which appears to be identical with our "diheterolevulosan IV". To avoid confusion we propose that our names "diheterolevulosan III" and "diheterolevulosan IV" should be interchanged.

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** All melting points uncorrected.