the α -direction is the same as the very much pronounced lath-direction of the crystals, mentioned above. The cleavage is also consistent with this arrangement. A somewhat similar hydrogen bond system has been found for formic acid³, but never before for a long chain carboxylic acid.

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Full details of this work will be published later.

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The Fractionation of Spruce Wood

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During the investigations on milled spruce wood, Björkman¹ was able to isolate material containing both lignin and carbohydrates and which was designated lignin-carbohydrate complexes (LCC).

It is proposed to report the preliminary results of further investigations on the extraction of wood with dimethyl sulphoxide (DMSO), of which brief mention has already been made².

The fractionation of milled spruce wood has been carried out by the successive extraction with dioxane, dimethyl formamide (DMF), DMSO and water, following which the cellulosic residues were recovered in yields of 41–43 %. These residues contained small amounts of lignin and hemicelluloses.

The extracts were fractionated at first by precipitation with selective solvents, but in an effort to avoid coprecipitation, later work involved distribution on columns of alumina, sand, or metallic oxides. However, when these were found to degrade the lignin, Celite columns were used instead. But in each case it was possible to isolate a series of lignin-carbohydrate complexes, together with relatively pure lignin and hemicellulose fractions. Except in one case where a small amount of a fraction containing glucose and mannose only (1:1.24) together with small amounts of lignin, was isolated from an alumina column, the LCC even where the lignin content was very small, still contained carbohydrates in the same approximate ratio as in the original hemicelluloses.

In one series of fractionations of milled spruce wood, the total amount extracted (31 %) was recovered almost entirely in the dioxane (9 %), DMF (7 %), DMSO (11.2 %) and the aqueous extracts (2 %). The difference is to be found in the DMSO mother liquors which were not further investigated in this series. The dioxane extract yielded a series of lignin fractions (CH_3O , 13–15 %) containing small amounts of carbohydrates, while the DMF extract gave a series of LCC (CH_3O , 5–12 % and lignin 10–90 %). Both the DMSO and the aqueous extracts could be largely precipitated by ethanol, yielding LCC material with 4 % CH_3O and about 22 % lignin. Whether the DMSO extract was precipitated by dioxane, ethanol or benzene, the complexes obtained still had methoxyl contents of about 3.5–4 % and lignin of about 22 %. Refractionations of these LCC gave a number of widely different fractions, with respect to their lignin content. Such fractionations were usually carried out on Celite columns with a combination of various solvent systems, *e. g.* ethyl acetate-acetic acid-water, butanol-pyridine-water or DMF-dinitromethane-water *etc.*

Intermediate lignin fractions, soluble in benzene and sometimes ether, were obtained in the first fractions from the Celite columns.

Since the relative proportion of the lignin to hemicellulose in the LCC modifies the solubility of the complex it was not surprising that LCC were isolated from clear solutions of benzene, dioxane, ethanol, water *etc.* Perhaps better methods for their isolation may be based on electrophoretic techniques.

Spruce wood chlorite holocellulose was also milled under the same conditions as for the wood, where it was found that extraction with DMF yielded about 10 % of a brown film, containing both modified lignin and hemicelluloses, while extraction with water gave about 22 % of a very white powder, which contained only hemicelluloses. The cellulosic residue was obtained in a yield of 43 %.

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Experiments with Methyl-fluorophosphorylcholine-inhibited Cholinesterase

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Organophosphorus cholinesterase inhibitors are considered to phosphorylate an esteratic site of the enzyme¹. It has been demonstrated that phosphorylated cholinesterase can be reactivated through dephosphorylation by hydroxylamine derivatives².

Methyl-fluoro-phosphorylcholines have been shown to be potent cholinesterase inhibitors and cholinergic drugs^{3,4}. It seemed thus to be of interest to investigate the possibilities of reactivating methyl-fluoro-phosphorylcholine-inhibited human erythrocyte acetylcholinesterase.

The inhibitors investigated were:

Methyl-fluoro-phosphorylcholine

Methyl-fluoro-phosphorylhomocholine

The reactivators investigated were: Pyridine-2-aldoxime methiodide (PAM)⁵; Nicotinic hydroxamic acid methiodide (NAM)⁶; Diisodonitrosoacetone (DINA)⁶; Diacetylmonoxime (DAM, Hopkin and Williams Ltd. Chadwell Heath Essex, Engl.)

The reactivators were solved in Michel's veronal buffer (pH = 8)⁷, the concentrations being 4×10^{-3} and 5×10^{-4} M. The experiments were carried out according to Davies and Green⁶.

Washed erythrocytes, diluted to blood volume with saline, were treated with an aqueous inhibitor solution (10^{-6} M) for 10 min at 25°C. The inhibited cell-suspension was centrifuged, the erythrocytes washed and diluted to the same volume as before. Equal volumes of this cell-suspension and the reactivator-buffer solution were mixed and stored at 25°C. At intervals between 10 and 60 min after incubation, samples were taken for enzyme activity determinations, which were carried out by means of an electrometric method⁷.

None of the reactivators reactivated the enzyme preparations, inhibited with any of the three methyl-fluoro-phosphorylcholines. However, as could be expected, Sarin-inhibited enzyme was reactivated by PAM⁸ in parallel runs.

The prevented reactivation is interesting because it may throw some further light on the nature of the inhibitor-enzyme reaction. An ionic bond in the inhibited enzyme, analogous to that postulated in the substrate enzyme complex⁹, and a shielding (Fig. 1) of the ionic site from the reactivator seem to explain the obstructed reactivation. The ionic bond increases the attraction of the organophosphoryl residue to the enzyme, which alone may be a sufficient explanation in case of DINA and DAM. In case of PAM and NAM

Methyl-fluoro-phosphoryl-β-methylcholine

Methyl-isopropoxy-phosphoryl fluoride, Sarin