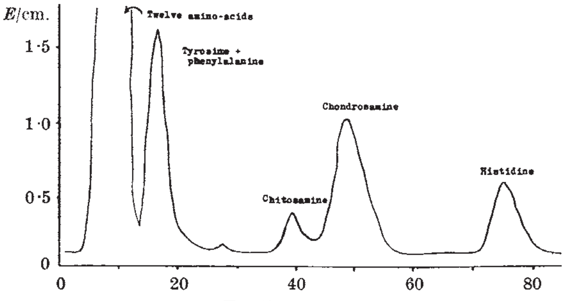

Fig. 1. A portion of a chromatogram of a protein - carbohydrate complex hydrolysate on a 15-cm. column of 'Dowex 50', showing separation of the hexosamines

liable to vary somewhat according to an unknown factor, possibly the flow-rate. Gardell[5] has separated glucosamine from galactosamine on 'Dowex 50' columns using 0·3 *N* hydrochloric acid as eluant, and Boas[6] used the same resin to remove interfering substances from hexosamine solutions. Both authors utilized modified Elson and Morgan[4] techniques to determine the hexosamines.

Recently, in connexion with the analysis of a protein – carbohydrate complex isolated from compact bone, we have succeeded in separating chitosamine and chondrosamine quantitatively on 15 cm. × 0·9 cm. columns of 'Dowex 50' using the standard procedure of Moore and Stein[1]. The positions of the peaks are shown in the accompanying figure, where the optical density of the solution, compared with water, is plotted against the fraction number. The chitosamine peak at fraction 40 is well separated from the chondrosamine peak at fraction 50. The hexosamine peaks are also separated from tyrosine and phenylalanine, from the amino-acids emerging before these and from the small peak at fraction 28, which is an artefact arising from the change of buffer at fraction 23 (0·1 *M* sodium citrate, *p*H 4·95 to 0·1 *M* sodium phosphate, *p*H 6·75). Histidine and hydroxylysine (not shown in the figure) emerge after the hexosamines and without overlap. The only known amino-acid emerging with the hexosamines is tryptophan, which is unlikely to cause difficulty in hydrolysates, since it is completely destroyed on heating with acid.

The complete separation of the hexosamines from amino-acids on the 15-cm. column enables the reliable ninhydrin procedure[3] to be used for estimation in place of the less accurate method of Elson and Morgan[4]. Convenience is also gained, compared with the use of the 100-cm. column, since a smaller number of fractions is required for the emergence of the amino-sugars. Both chitosamine and chondrosamine give the same colour intensities with ninhydrin as an equimolecular weight of leucine, that is, colour yield is 1·00[3]. Both hexosamines were recovered quantitatively from the column, when added as the pure hydrochlorides. The method, therefore, provides an accurate means of determining small quantities of chitosamine and chondrosamine in hydrolysates of complex polysaccharides, whether or not proteins are also present. The decomposition of hexosamines, in the presence of amino-acids, under the initial hydrolysis conditions has not been fully investigated. More than 98 per cent of the total nitrogen, as shown by the ninhydrin-positive components, was recovered from a protein – carbohydrate complex, which contained 8 per cent of hexosamines and more than 60 per cent of amino-acids, after 0·05 gm. was hydrolysed with 10 ml. of 20 per cent w/w hydrochloric acid at 100° for 24 hr. It would thus appear that the amount of breakdown is small when a large excess of acid is used for hydrolysis.

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Cleavage of the 2 : 4-Dinitrophenylhydrazones of Ketones

THE interesting contribution of J. Demaecker and R. H. Martin[1] on this subject prompts me to mention an alternative method which was devised in the course of work (1946) in the strychnine series.

The ketone 4-nitrophenylhydrazone or 2 : 4-dinitrophenylhydrazone was heated on the steam-bath with 80 per cent formic acid (or stronger) and copper carbonate added. The end-point was in most cases indicated by the disappearance of colour. In this process the nitro- or dinitro-phenylhydrazine produced by hydrolysis is destroyed by oxidation, and, however unfavourable the equilibrium between hydrazine and hydrazone may be, the liberation of ketone goes to completion. The method was found to be generally applicable, and the yields of ketone were high.

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Preparation of Monodeuteriomethyl Bromide

THE exchange of deuterium and hydrogen has prevented the attainment of a high degree of isotopic purity in the preparation of compounds of the type CH_2DX, where X is a halogen atom or hydroxyl group. CH_2DOD has been prepared by the action of diazomethane on heavy water. Halford *et al.*[1] acidified the D_2O with SO_3. No exact indication of the isotopic purity of the product was obtained[2]. Langseth and Bak[3] claimed that about 10 per cent CH_3OH was present in CH_2DOD prepared by the same method due to water unavoidably present in the CH_2N_2. Beersmans and Jungers[4] repeatedly bubbled gaseous CH_2N_2 through D_2O until reaction was complete. The product was a mixture of all the different isotopic forms. Without acidification the reaction may proceed by a free radical mechanism[5] leading to exchange. The methyl bromide prepared by esterification with HBr (in which no exchange occurs) contained 51 per cent CH_2DBr and CHD_2Br [6].

I prepared CH_2DBr in about 93 per cent isotopic purity by the direct action of CH_2N_2 and DBr in dibutyl ether[7]. The reagents are difficult to handle on a large scale, and it does not seem possible to avoid exchange with such reactive materials.

CH_2DBr has now been prepared in high yield by the method:

$$CH_2CO + D_2O \rightarrow CH_2D.COOD$$

$$CH_2D.COOD + CH_2CO \rightarrow (CH_2D.CO)_2O$$

$$(CH_2D.CO)_2O + Ag_2O \rightarrow 2\ CH_2D.COOAg$$

$$CH_2D.COOAg + Br_2 \xrightarrow{CCl_4} CH_2DBr + CO_2 + AgBr.$$

Ketene was prepared in the standard way[8] and bubbled directly through D_2O. The acetic anhydride, after fractional distillation, combined directly with dry Ag_2O. The method of Bockmuller and Hoffman[9] was followed in the bromination.

Mass-spectrometric analysis indicated about 5 per cent CH_3Br in the product. This agreed with the analysis of the anhydride and indicated that exchange did not occur in the last steps of the reaction.

Langseth and Bak[3] prepared $CH_2D.COOCH_3$ in high isotopic purity by partial acid hydrolysis of dimethyl malonic ester in D_2O followed by decarboxylation of the resultant mono-ester. Silver acetate was prepared from the acid, and the methyl bromide produced by bromination as above was a mixture of CH_3Br, CH_2DBr, CHD_2Br and CH_3Br. The exchange was presumed to occur in the last step.

In view of the above results, it is more probable that water, formed in the action of acetic acid on silver oxide, was exchanged with the methyl group of the silver acetate[10,11].

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Properties of the Calcium Phosphates

WORKERS on the chemistry of soils and fertilizers have to deal with phosphorus compounds, many of which are complex or of indefinite composition. It is likely that, if fundamental information were available concerning those compounds of fairly well-defined composition, the transformations occurring during manufacture and after application to the soil might be more understandable.

The most widely used phosphate fertilizer is superphosphate, which consists mainly of monocalcium phosphate and calcium sulphate. It depends for its production upon the use of large quantities of sulphuric acid. Past shortages and doubts concerning future supplies of this acid are among the reasons focusing attention on fertilizers containing more basic phosphates such as dicalcium phosphate, hydroxyapatite and materials of composition between these.

A co-operative programme of work has been commenced in these laboratories aiming in the first instance at establishing the structures of certain calcium phosphates and at developing laboratory techniques permitting an approximate evaluation of their agronomic value.

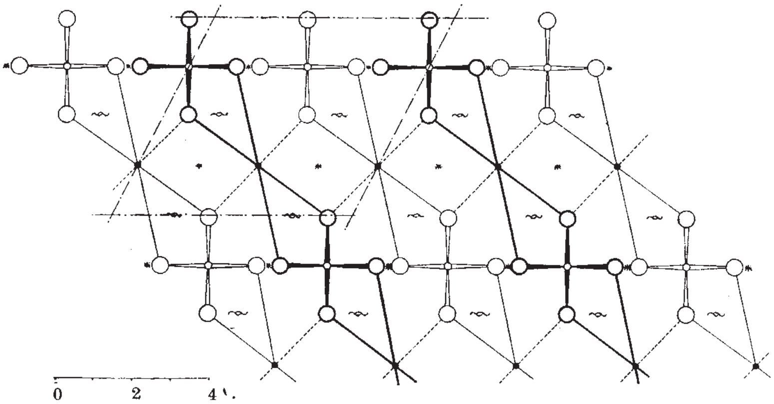

Fig. 1. A sheet of composition $CaPO_4$ in the structure of $CaHPO_42H_2O$

The structures being studied at present are those of $CaHPO_42H_2O$ and $CaHPO_4$. A forecast that the former structure would be similar to that of gypsum has already been made[1], and we have confirmed the correctness of this by single-crystal X-ray methods.

The unit cell dimensions of $CaHPO_42H_2O$ are: a = 5·812, b = 15·180, c = 6·239 A., β = 116° 25′. These data correspond to a different choice of a-axis (and of β) from that used by Wooster for gypsum[2]. Wooster's values were apparently taken from Onorato[3]. The space group of $CaHPO_42H_2O$ is I 2/a and the cell contains four formula units. The Ca and P (and probably the H also) are on the two-fold axes, the y parameters of Ca and P differing by exactly a half. These positions of the heavy atoms enable a complete structure determination to be carried out by Fourier methods, and this work has now been almost completed.

In Fig. 1 (a projection along the b-axis), PO_4 groups are represented by oxygen atoms arranged tetrahedrally around the central phosphorus atom. The calcium atoms have six of their eight co-ordination positions occupied by oxygen atoms from PO_4 groups and the remaining two are filled by water molecules. The basic structure can conveniently be regarded as being built of sheets containing parallel chains of composition $=PO_4=Ca=PO_4=Ca=$. These run down from left to right in Fig. 1. Adjoining chains in any one sheet differ in height by 2·3 A. to give a corrugation effect. The higher set of chains is related to the lower by centres of symmetry between the PO_4 groups and between the calcium ions of different chains. The corrugated sheets themselves are repeated at a separation of $b/2$ = 7·59 A. by vertical screw axes. Between the sheets are the water molecules, each molecule linking a calcium atom and an oxygen atom in one sheet to an oxygen atom in an adjacent sheet.