55.4; O-acetyl, 31.7. Found: C, 49.45, 49.71; H, 6.51, 6.61; N, 7.65; total acetyl, 53.4; O-acetyl, 30.2; mol. wt. (Rast, in camphor), 543, 512.

(2) Non-identical methyl glycosidic moieties, which for reasons given below have been provisionally termed methyl neobiosaminides B and C ($[\alpha]_D$ of the amorphous hydrochlorides, 1.0% in water, +22° and +78°, respectively). The analysis of a chromatographically purified amorphous *polyacetate of methyl neobiosaminide B* was best compatible with the composition $C_{11}H_{16}O_6N_2(OCH_3)(COCH_3)_5$: $[\alpha]^{22}D$ +2°, 0.5% in ethanol. *Anal.* Calcd.: C, 50.96; H, 6.61; N, 5.40; CH_3O, 5.98; 5 acetyl, 41.5. Found: C, 49.62; H, 6.70; N, 5.41; CH_3O, 5.16; acetyl, 41.9.

On vigorous hydrolysis with hydrochloric acid, methyl neobiosaminide C yielded the dihydrochloride of a reducing diamine, $C_6H_{14}O_3N_2.2HCl$, platelets from methanol–ether; m.p. 182–185° with decomposition after shrinking and darkening 155–175°, $[\alpha]^{22}D$ +69°, equilibrium value, 0.4% in water. *Anal.* Calcd.: C, 30.65; H, 6.86; N, 11.92; Cl, 30.16. Found: C, 30.79; H, 7.18; N, 12.52; Cl, 30.51. The reducing diamine from methyl neobiosaminide B has not yet been obtained in crystalline form but mutarotation and paper chromatographic data indicate that it is not identical with that from neomycin C. The remainder of the neobiosaminide fragment seems to be accounted for by a pentose, as evidenced by the formation, on acid hydrolysis, of furfural from both neomycin B and C and the corresponding methyl neobiosaminides. This property has been utilized for the chemical assay of the two antibiotics.

The average weight ratio, in several methanolyses, of non-glycosidic fraction to methyl biosaminide fraction was 1.85; on the other hand, the ratio of the molecular weight of the C_9 base hydrochloride (358) to that of the methyl biosaminide hydrochloride (381) is 0.94. Hence it appears that actually two moles of the C_9 base are liberated, conceivably from acetalic linkage, by the methanolysis. On this basis the neomycins B and C would appear to have the composition $C_{29}H_{58}O_{16}N_8$.

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A TERMINAL AMINO ACID RESIDUE OF LYSOZYME AS DETERMINED WITH 2,4-DINITROFLUOROBENZENE

Sir:

Studies on lysozyme to determine which amino acid residues occupy terminal positions at the amino ends of the polypeptide chains have shown that one of the lysine residues occupies such a position in the protein. This conclusion is drawn from the fact that α,ε-di-DNP-lysine was the only DNP-amino acid isolated chromatographically from the ether extract of an acidic hydrolysate of DNP-lysozyme. No attempt has yet been made to determine whether the hydrolysate contains basic DNP-amino acids other than ε-DNP-lysine which are not extracted with ether.

DNP-Lysozyme was prepared from lysozyme (Armour and Company Lot 805L1) in a manner similar to that described by Sanger[1] for insulin. A sample of the DNP-lysozyme was refluxed with 6 *N* hydrochloric acid for 8 hours and the ether extract of the hydrolysate was examined chromatographically according to a scheme which has recently been developed in these Laboratories.[2] This scheme for the separation of 16 ether-soluble DNP-amino acids by adsorption chromatography on 2:1 silicic acid–Celite is considerably faster than that described by Sanger,[1] Porter and Sanger,[3] and Blackburn,[4] and gives satisfactory qualitative results on a large number of samples of silicic acid. The ether extract of the hydrolysate gave two yellow zones on a silicic acid column. The chromatographic characteristics of the lower zone were identical with those of α,ε-di-DNP-lysine with three different solvent systems as developers, even after a second hydrolysis. The upper zone, which did not behave like any known DNP-amino acid, yielded α,ε-di-DNP-lysine on further hydrolysis and, therefore, contained a DNP-lysyl peptide (or peptides), highly resistant to hydrolysis, which is now being investigated. The changes in development behavior of α,ε-di-DNP-lysine from one developer to another are greater than those of other similarly adsorbed DNP-amino acids so that its chromatographic behavior is an excellent criterion for its identity.

In preliminary quantitative studies it has been found that the ether extract of an 8-hour hydrolysate contained α,ε-di-DNP-lysine equivalent to about 0.35 terminal amino groups per molecule (mol. wt. 13,900[5]), and in addition a considerable amount of DNP-lysyl peptide(s). After hydrolysis for 24 hours, α,ε-di-DNP-lysine equivalent to 0.75 terminal amino groups per molecule was isolated. This value, which includes corrections for 7% loss during chromatographic operations and for 15% destruction of the lysine derivative during hydrolysis, is considerably less than one terminal group per molecule and further improvements in the analytical method are now being sought. There was still evidence of a small amount of DNP-lysyl peptide(s) after 24 hours hydrolysis but probably not enough to account for the low yield of lysine derivative. The absence of free lysine when the amino acid content of the hydrolysate was determined by starch chromatographic methods indicates the completeness of the reaction between the protein and dinitrofluorobenzene. From the results obtained thus far it seems unlikely that more than one lysine residue occupies an amino terminal position in lysozyme.

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(1) F. Sanger, *Biochem. J.*, **39**, 507 (1945).
(2) F. C. Green and L. M. Kay, to be published.
(3) R. R. Porter and F. Sanger, *Biochem. J.*, **42**, 287 (1948).
(4) S. Blackburn, *ibid.*, **45**, 579 (1949).
(5) K. J. Palmer, M. Ballantyne and J. A. Galvin, THIS JOURNAL, **70**, 906 (1948).