added to the aqueous filtrate and concentrated to a syrup *in vacuo*. The concentrate is dissolved in methanol and diluted with excess of ethyl acetate, and the precipitate removed. The solvents are evaporated and the residue is dissolved in methanol and fractionated on a column of Sephadex LH-20 with methanol as eluent. The fractions showing biological activity are pooled and the solvent is removed. The residue is dissolved in water and passed down a column of Amberlite IR-120 cation-exchange resin; the aqueous eluate is evaporated to a pale-yellow viscous residue and dissolved in methanol for chromatography. Chromatography is done on Merck micro-crystalline cellulose plates in butan-1-ol-acetic acid-water (12:3:5, by vol.). After development, the band running at R_F 0.45 is removed, packed into a small column and eluted with methanol. On removal of the solvent a clear colourless gum remains, which has crystallized on occasions to give feathery white crystals, m.p. 110°C.

Mass spectrometry indicates a parent ion of m/e 156, molecular formula $C_7H_8O_4$, and implies the presence of four double bonds or their equivalent. The spectra suggest a furan derivative, and there is evidence for a $-CH_2-CO_2H$ group. The n.m.r. spectrum showed the absence of furan ring protons and of CH_3- or $-CH_2-$ groups that are not subject to a strong downfield shift by other groups. The spectrum consists of a broad hump at 5.5 p.p.m. with two sharper peaks coincident with it; these absorptions are in the olefinic region.

The i.r. spectrum shows the following peaks: 3340 ($-OH$), 2860 (alkyl) and 1690–1700 cm^{-1} ($=CO$). The u.v. spectrum shows only end-absorption at 205–210 nm.

The isolated material is very soluble in water, less so in methanol; solutions have only a limited stability, even at $-20^\circ C$. It appears to polymerize when heated under acid conditions; alkali causes complete loss of activity. There is some evidence that the substance can be formed by very mild hydrolysis of the precipitate from the ethyl acetate fractionation (Leach, 1970). The substance is acidic, and gives a positive reaction for lactones and/or esters. It decolorizes neutral aqueous potassium permanganate, and this reaction has been used for locating the bands on t.l.c. plates; reactions for aldehydes and ketones are negative (Tollens reagent and 3-methylbenzylthiazolone).

Mutagenic and carcinogenic activity are shown by the isolated material; after intraperitoneal injections (10 mg) to mice death occurs within a few hours, with haemorrhages and denudation of the intestinal mucosa. With 2–3-month-old calves fractions are active up to the ethyl acetate supernatant stage; the formidable task of preparing enough of the material isolated by t.l.c. for trial on

the bovine animal has not yet been attempted. Possible structures for the bracken toxin will be discussed.

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Alkaline Reductive Cleavage of 4-N-(2-Acetamido - 2 - deoxy - β - D - glucopyranosyl) - L - asparagine

By B. M. AUSTEN and R. D. MARSHALL. (*Department of Chemical Pathology, St Mary's Hospital Medical School, London W.2, U.K.*)

Treatment of glycoproteins, which contain O-glycosidic linkages to serine and threonine residues, with alkaline sodium borohydride was introduced to obtain reduced oligosaccharides (Schiffman, Kabat & Thompson, 1964) and to examine the amino acid residues forming the point of attachment of the carbohydrate prosthetic groups (Tanaka, Bertolini & Pigman, 1964). Present results suggest that similar procedures might be applied to the preparation of reduced oligosaccharides from glycoproteins or glycopeptides that contain 4-N-(2-acetamido - 2 - deoxy - β - D - glucopyranosyl) - L - asparagine (I) as a carbohydrate-peptide linking moiety. Studies of the products formed when compound (I) and 4-N-(β -D-glucopyranosyl)-L-asparagine (II) are treated with hot aqueous alkali, both in the presence and in the absence of sodium borohydride, have been carried out.

First-order rate constants for the destruction of compounds (I) and (II) in 0.2M-sodium hydroxide at $100^\circ C$ were 6.44×10^{-3} and $5.97 \times 10^{-3} min^{-1}$ respectively. The rate of release of ammonia under these alkaline conditions from compound (I) followed complex kinetics, with an initial lag phase. Approx. 1.5 mol of ammonia was produced after a time equivalent to five half-lives for destruction of compound (I). U.v.-absorbing material was formed (λ_{max} , 264 nm) with a minimum molar extinction coefficient of 1300, this value being based on the concentration of compound (I). L-Aspartic acid was formed from compounds (I) and (II) with first-order rate constants of about 2.8×10^{-3} and $2.6 \times$

10^{-3} min^{-1} respectively. Thus after five half-lives for the reactions in which compounds (I) and (II) are destroyed only about 45% of the theoretical amount of aspartic acid had been released in each case. Free L-aspartic acid was found to be stable under the conditions employed, both in the presence and the absence of free N-acetyl-D-glucosamine.

In 0.05M-sodium hydroxide at 100°C the first-order rate constants for the destruction of compound (I) and for the formation of aspartic acid from it were 3.1×10^{-3} and $0.87 \times 10^{-3} \text{ min}^{-1}$ respectively. These data and those obtained in 0.2M-sodium hydroxide may suggest that the reaction whereby compound (I) is destroyed without formation of aspartic acid is intramolecular. N-Deacetylated compound (I) is unlikely to be the major product in which the aspartic acid that is not released is incorporated, since acid hydrolysis of the alkali-treated material does not release aspartic acid except from undegraded compound (I).

Compound (I) was destroyed at rates of $21 \times 10^{-3} \text{ min}^{-1}$ and $11 \times 10^{-3} \text{ min}^{-1}$ at 100°C in 0.2M- and 0.05M-sodium hydroxide respectively in the presence of 1M-sodium borohydride. Approx. 60 and 50% of compound (I) respectively were degraded to give aspartic acid with, in each case, similar amounts of reduced sugars. Substances with the chromatographic mobilities of asparagine and of homoserine are formed in the alkaline reductive cleavage of compound (I). The identities of these amino acids were confirmed from thin layer chromatographic studies of their dansyl derivatives.

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The Determination of Methionine by Gas-Liquid Chromatography

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Under certain conditions peptide-bound methionine is sensitive to oxidation to the sulfoxide. Such oxidations may be intentional and specifically directed to establish the function of individual methionine residues in enzyme activity (Neumann, Moore & Stein, 1962), or they may be inadvertently incurred during some protein isolation procedures (Grant & Reid, 1968). A rapid method has been developed to monitor the extent of oxidation of methionine residues in proteins. It is based on the observation that, whereas the first stage of the modified Witkop reaction of cyanogen bromide with proteins is quantitative for methionine (Schroeder, Shelton & Shelton, 1969; Inglis &

Edman, 1970), yielding methyl thiocyanate, methionine sulfoxide does not react.

The protein was dispersed in concentrated formic acid before overnight reaction in 70% (v/v) formic acid with twice its weight of cyanogen bromide. 4-Methylcyclohexanone was added as an internal standard before the reaction mixture was adjusted to a final concentration of 30% (v/v) formic acid. Methyl thiocyanate was determined by g.l.c. and flame ionization in a Hewlett-Packard model 5750B analyser. The reaction of cyanogen bromide with free methionine took appreciably longer than that with the peptide-bound form, but this too was completed during the overnight incubation.

The method was applied to the measurement of the methionine content of lysozyme, ovalbumin, bovine serum albumin, β -lactoglobulin and two samples of casein. Results were compared with those obtained by the Moore & Stein analysis for methionine sulphone after performic acid oxidation (Moore, 1963). The two methods mostly agreed well, but the g.l.c. value for lysozyme was 7% low and that for β -lactoglobulin 7% high. Samples of casein oxidized by hydrogen peroxide (Ellinger & Palmer, 1969) were also examined. Their methionine sulfoxide content determined after alkaline hydrolysis (Neumann *et al.* 1962) corresponded to the loss of methionine as measured by the g.l.c. method.

Various reducing agents are being examined for their effectiveness in the conversion of the sulfoxide into methionine.

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Conjugates of Phenylacetic Acid with Taurine and other Amino Acids in Various Species

By M. JAMES, R. L. SMITH and R. T. WILLIAMS. (Department of Biochemistry, St Mary's Hospital Medical School, London W.2, U.K.)

Phenylacetic acid and certain other arylacetic acids are excreted in man and certain monkeys as glutamine conjugates, whereas in lower animals they form glycine conjugates (see Williams, 1967). An examination of the fate of [^{14}C]phenylacetic acid