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IDENTIFICATION BY MASS SPECTROMETRY OF N-β-ASPARTYL- AND N-γ-GLUTAMYL-2-DEOXY-2-ACETAMIDO-β-D-GLUCOSYL AMINE DERIVATIVES (*)

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It has been reported that the carbohydrate-peptide linkage in ovalbumin (1 - 4), ovomucoid (5, 6), γ-globulin (7 - 9), α_1-acid glycoprotein (10 - 12) and fibrinogen (13, 14) is formed by N-β-aspartyl-2-deoxy-2-acetamido-D-glucosylamine. The structure of this compound has been confirmed by synthesis (15, 16). However, the occurence of other similar linkages, formed e.g. by D-glucosamine (or D-galactosamine) and glutamine, is also possible.

The aim of the present work is to develop a general method for the detection and identification of small quantities of N-acyl-2-deoxy-2-acetamido-β-D-hexosylamine derivatives. The hexosamine-aminoacid linkage such as exists in these compounds represents a type of carbohydrate-peptide linkage which is thought to be of general occurence in a large variety of glycoproteins.

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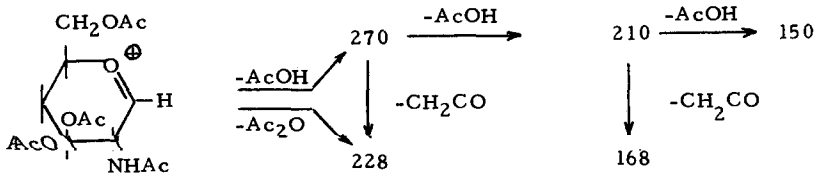
With this in view, we investigated by mass spectrometry (*) three synthetic derivatives :

(a) - N-(α-Benzyl N-benzyloxycarbonyl-L-γ-glutamyl)-2-deoxy-2 acetamido-3,4,6-tri-O-acetyl-β-D-glucosylamine (17) (I), whose structure and mass spectrum are shown in figure 1.

(b) - The corresponding L-β-aspartyl derivative (17) (II).

(c) - N-(α-Ethyl N-trifluoracetyl-L-β-aspartyl)-2-deoxy-2-acetamido-3,4,6-tri-O-acetyl-β-D-glucosylamine (III). The structure and mass spectrum of this last compound (m.p. 252-5°), obtained by condensation of 2-deoxy-2 acetamido-3,4,6-tri-O-acetyl-D-glucosylamine with N-trifluoracetyl-L-aspartic acid α-ethyl ester in the presence of dicyclohexylcarbodiimide, is shown in figure 2.

The amino-sugar moiety of the compounds is indicated in the spectra by a fragment at m/e 330 :



(*) - The spectra were recorded with an high resolution instrument MS9 (A.E.I.). The temperature in the ion source was about 280°C, the electron energy was set at 70 eV. The samples were introduced directly into the ion source throught a vacuum lock system.

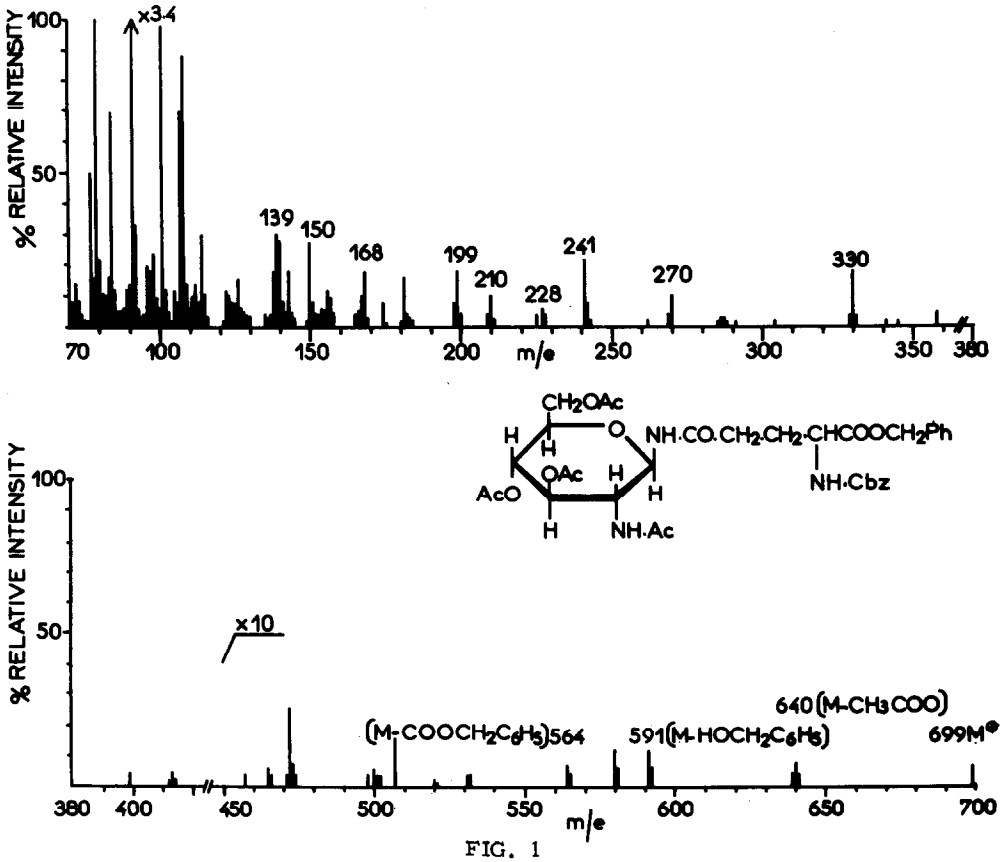

% RELATIVE INTENSITY
100
50
x3.4
139
150
168
199
210
228
241
270
330
70
100
150
200
m/e
250
300
350
380
CH2OAc
H
H
O
NH.CO.CH2.CH2.CHCOOCH2Ph
OAc
H
AcO
H
NH.Cbz
H
NH.Ac
% RELATIVE INTENSITY
100
50
x10
640(M-CH3COO)
(M-COOCH2C6H5)564
591(M-HOCH2C6H5)
699M⊕
380
400
450
500
550
m/e
600
650
700

FIG. 1

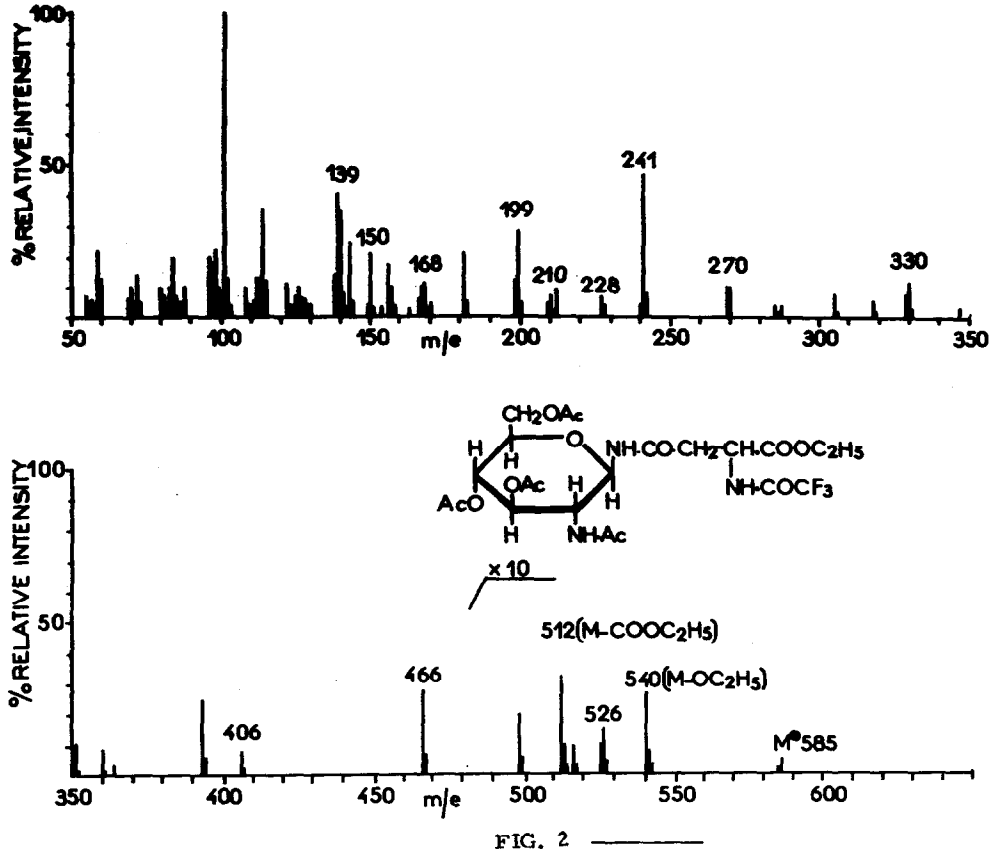
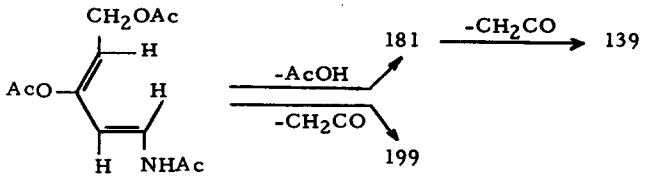

% RELATIVE INTENSITY
100
50
139
150
168
199
210
228
241
270
330
50
100
150
m/e
200
250
300
350
CH2OAc
O
NH·CO·CH2·CH·COOC2H5
NH·COCF3
AcO
OAc
H
NHAc
x 10
512(M-COOC2H5)
466
540(M-OC2H5)
526
406
M⊕585
350
400
450
500
550
600

FIG. 2

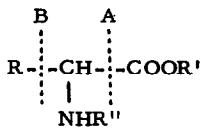
Further fragmentation of the oxonium ion corresponds to the general reactions observed in peracetylated sugars (18) and peracetylated amino-sugars (19), involving the loss of acetic acid and ketene ; it may be summarized by the above scheme. The fragments shown in the scheme may be accompanied by ions containing one less hydrogen atom which originate from an ion at m/e 329.

Another peak series is found starting from an ion at mass 241, which corresponds to the structure of the ion at m/e 242 in peracetylated sugars (18). The loss of acetic acid and ketene from this ion is outlined in the following scheme :



m/e 241

The molecular ions are found for (I) at m/e 699, for (II) at m/e 685 and for (III) at m/e 585. Characteristic peaks of most amino-acid esters are due to the loss of the ester group (20) (cleavage A) :

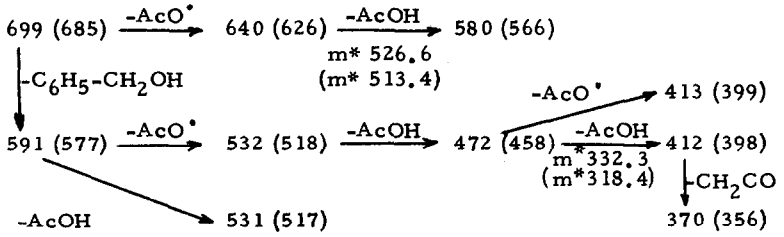


Peaks corresponding to cleavage A are detected in (I) at m/e 564, in (II) at m/e 550 and in (III) at m/e 512, which permits an unequivocal determination of the amino-acids to be glutamic acid in (I) and aspartic acid in (II) and (III).

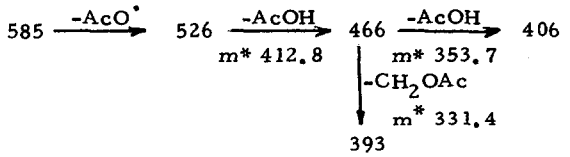
In the benzyloxycarbonyl derivatives (I) and (II), one of the most intense peaks in the high mass region (m/e 591 for (I) and 577 for (II)) is due to the thermal elimination of benzyl alcohol. This finding is in agreement with the recently reported (21) loss of benzyl alcohol in the ion source of the mass spectrometer which was found to occur predominantly above a sample temperature of 200°C.

A simplified breakdown pattern of (I) and (II) is outlined in the scheme (a),of compound (III) in scheme (b).

Scheme (a) (*)



Scheme (b)



On the basis of these observations mass spectrometry is shown to be a suitable method for the identification of hexosamine-aminoacid linkages in N-acyl-2-deoxy-2-acetamido-hexosylamine derivatives, thus allowing identification of similar linkages in glycopeptides. Determination of the aminoacid

(*) - Data in parantheses are for compound (II).
m* indicates metastable peaks.

sequence in the environment of these linkages in glycopeptides should then be feasible. The work is now being extended to natural products.

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