[CONTRIBUTION FROM THE DIVISION OF NUTRITION AND PHYSIOLOGY, BUREAU OF DAIRY INDUSTRY, U. S. DEPARTMENT OF AGRICULTURE]

# The Determination of Methylpentoses in the Presence of Pentoses

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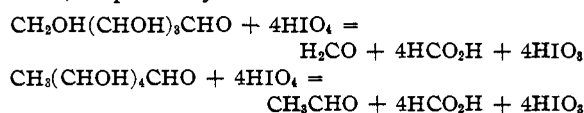
Methylpentoses (6-desoxy-hexoses) may be determined with moderate accuracy by existing methods when pentoses are absent. But this condition is probably rarely fulfilled in those natural products the study of which would be most interesting. All methods usually considered are based on that of Ellet and Tollens,[1] and estimate by various procedures the amount of methylfurfural formed by distillation with hydrochloric acid under rigidly standardized conditions. Highly empirical calibration curves are ordinarily necessary to interpret the results.

In spite of a large amount of earnest effort by many workers, no method for the determination of any methylpentose in the presence of pentoses, even in synthetic mixtures, is even approximately satisfactory.

As the method we wish to describe has nothing in common with previous methods, there is no occasion to discuss these in detail. Of recent papers, those of Hughes and Acree[2] and of Marshall and Norris[3] will point the way to earlier references desired.

Periodic acid should react with a pentose or a methylpentose according to the following equations, respectively



$$CH_2OH(CHOH)_3CHO + 4HIO_4 = H_2CO + 4HCO_2H + 4HIO_3$$

$$CH_3(CHOH)_4CHO + 4HIO_4 = CH_3CHO + 4HCO_2H + 4HIO_3$$

The hexose reaction would be analogous to that for pentoses.

As these reactions are sufficiently quantitative, the problem of determining methylpentoses in the presence of other sugars (including pentoses) becomes essentially the problem of determining acetaldehyde quantitatively in the presence of formaldehyde. This problem we have recently solved (so far as we know, for the first time) in connection with recently published methods for the determination of threonine[4] and serine[5] in protein hydrolyzates. The results were very satisfactory.

In an attempt to apply this separation more widely, we have found it possible to determine moderately small quantities (10 mg.) of rhamnose in the presence of a pentose and a hexose with considerable accuracy, and without the aid of any correction factor. We believe that this has not been done before, and we see no reason why the *nature* of a particular pentose or a particular hexose should influence the results.

Table I will illustrate the results. The values given in Columns 2, 3, and 4 are factual and illustrative, but somewhat wide variations are permissible.

It should also be noted that accurate results

TABLE I

RECOVERY OF METHYL PENTOSES AND DERIVATIVES, ALONE AND IN MIXTURES

| Sample | $NaHCO_3$ ($N$), cc. | Arsenite (0.1 $N$), cc. | $HIO_4$ (0.5 $M$), cc. | Methyl pentose found, % |
|---|---|---|---|---|
| 20 mg. rhamnose[a]<br>200 mg. alanine | 5.0 | 10.0 | 2.3 | 97.8 |
| 10 mg. *l*-fucitol[a]<br>50 mg. alanine | 5.0 | 10.0 | 1.5 | 97.7 |
| 10 mg. rhamnose[a]<br>10 mg. xylose<br>10 mg. glucose<br>200 mg. alanine | 7.0 | 10.0 | 2.5 | 99.2 |
| Same as above | 7.0 | 10.0 | 2.5 | 98.5 |
| 10 mg. rhamnose[a]<br>110 mg. xylose<br>10 mg. glucose<br>200 mg. alanine | 12.0 | 10.0 | 7.0 | 99.4 |
| 4 mg. rhamnose<br>4.34 mg. glucose<br>1 mg. galacturonic acid[b] | 5.0 | 10.0 | 2.0 | 100.0 |
| Same as above[b] | 5.0 | 10.0 | 2.0 | 98.5 |

[a] Rhamnose, wherever specified, was weighed as the monohydrate. The rhamnose and *l*-fucitol used were supplied by Dr. C. S. Hudson and Dr. R. M. Hann of the National Institute of Health, to whom we are most grateful. [b] We wish to thank Dr. Max Phillips, of the Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture, both for the galacturonic acid used, and for advice as to the most interesting conditions of acid treatment. These samples were refluxed for six hours with 1.5% sulfuric acid before analysis.

(1) W. B. Ellet and B. Tollens, *Ber.*, **38**, 492 (1905).

(2) E. E. Hughes and S. F. Acree, *Ind. Eng. Chem., Anal. Ed.*, **9**, 318 (1937); *J. Research Natl. Bur. Standards*, **21**, 327 (1938); **23**, 293 (1939).

(3) C. R. Marshall and F. W. Norris, *Biochem. J.*, **31**, 1053, 1289, 1939 (1937).

(4) L. A. Shinn and B. H. Nicolet, *J. Biol. Chem.*, **138**, 91 (1941).

(5) B. H. Nicolet and L. A. Shinn, *ibid.*, in press (1941).

are recorded for *l*-fucitol, which might be considered as evidence that the *configuration* of monosaccharides is not a factor which need cause interference.

We have also been able to show that heating with sulfuric acid under conditions ordinarily considered sufficient to hydrolyze glycosides or polysaccharide combinations not approaching too closely the cellulose type, does not interfere with the application of the method. The last two rows of Table I, in connection with Note (b), should adequately illustrate this.

Finally, the application to a natural product seemed to be in order. A sample of seaweed (6) was hydrolyzed for six hours by refluxing with 4% sulfuric acid. There is a presumption that some of the acid was neutralized by calcareous adhesions to the sea weed.

Table II shows that the results obtained are highly consistent. Inasmuch as there was a large residue of insoluble material at the end of *each* hydrolysis, it was surprising that the results from successive hydrolyses agreed so well. It was also a surprise when a *re-hydrolysis* of one of the residues gave results for fucose less than the normal variation of 0.2% in the reported value.

TABLE II

THE DETERMINATION OF FUCOSE IN SEAWEED[6]

| Hydrolysis | Sample represents seaweed, mg. | Alanine, mg. | $NaHCO_3$ (*N*), cc. | Arsenite (0.1 *N*), cc. | $HIO_4$ (0.5 *M*), cc. | Fucose, % |
|---|---|---|---|---|---|---|
| I | 100 | 200 | 5.0 | 12.0 | 1.8 | 10.34 |
| I | 80 | 200 | 5.0 | 10.0 | 1.5 | 10.40 |
| II | 100 | 200 | 5.0 | 12.0 | 1.8 | 10.02 |
| II | 80 | 200 | 5.0 | 10.0 | 1.5 | 10.06 |
| III | 100 | 200 | 5.0 | 12.0 | 1.8 | 10.19 |
| III | 80 | 200 | 5.0 | 10.0 | 1.5 | 10.35 |

We believe that we have already shown the method here described for methylpentoses to be far superior to any previous method. It has, however, another virtue. We have formerly shown that acetaldehyde and formaldehyde may, under certain conditions, be determined on the same sample.

Using the technique described in the papers cited, it has been possible to determine, in addition to fucose, "other carbohydrates" in the hydrolyzate, since formaldehyde could be independently estimated. These amounted to 6.6 or 5.5%, according to whether they were calculated as hexoses or pentoses.

## Experimental

The **apparatus** consists essentially of three Pyrex test-tubes (2.5 × 20 cm.) fitted as a gas absorption train; except that Tube I carries a dropping funnel, the stem of which reaches nearly to the bottom of the tube, and serves in this case as the gas inlet tube.

The acetaldehyde is produced in the first tube, and absorbed in the second and third.

It is useful to have a flow meter to gage the rate at which carbon dioxide is passed during the determination.

The **reagents** requiring reference are: (1) sodium arsenite, about 0.1 *N*, containing 20 g. sodium bicarbonate per liter; (2) periodic acid, about 0.5 *M*; (3) sodium bisulfite, "2%," containing 19 g. of metabisulfite per liter.

**Method.**—Before starting the reaction, the three tubes are charged as follows.

Into the reaction tube (I) are introduced, in the order indicated, (1) the sample, which should preferably contain 5–15 mg. of methylpentose, and in a volume not much exceeding 5 cc.; (2) about 0.2 g. of alanine; (3) one drop of Nujol to prevent foaming, unless the sample has been decolorized for the determination of "other carbohydrates"; (4) 5 cc. of *N* sodium bicarbonate[7]; (5) 10 cc. of 0.1 *N* sodium arsenite.[8]

Tubes II and III should contain, respectively, 5 cc.[9] and 3 cc.[9] of sodium bisulfite, diluted in each case to 25 cc.

The apparatus is then connected to a source of carbon dioxide, and gas passed for several seconds to mix the contents of all the tubes. The train is then broken, to introduce into the funnel (stopcock closed) the required amount[10] of periodic acid.

The system is reconnected, the stopcock of the funnel opened, and the periodic acid solution allowed to flow in under carbon dioxide pressure.[11] Carbon dioxide is passed for one hour at about 1 liter per minute.

At the end of this period, the contents of tubes II and

(6) *Fucus ascophyllum nodosum*, kindly supplied by Dr. H. S. Isbell of the National Bureau of Standards. "The seaweed, supplied by the Marine Laboratory at Woods Hole, Mass., was merely washed with tap water, air dried, and ground." We kept the sample in a vacuum desiccator for two weeks, before use.

(7) If quite large amounts of other carbohydrates are present, this must be increased to 10 cc. or more, as much formic acid is produced. An excess does no harm.

(8) The arsenite *must* be sufficient to reduce all the periodate *not* reduced by reaction with sugars. This amount is usually adequate.

(9) Neither the volume nor the concentration need be precisely known.

(10) Often 1–2 cc. is sufficient. In order to determine the amount required for a *completely* unknown sample, proceed as follows: A sample of the size to be used is neutralized (to litmus) with bicarbonate, and 10 cc. excess bicarbonate added; 2 cc. of 0.5 *M* periodic acid is added, the solution mixed, and allowed to stand for two minutes. Then 2 cc. of 20% potassium iodide is added, and the iodine liberated is titrated with standard arsenite. The arsenite is equivalent, mole for mole, to the periodic acid excess (if there *is* no excess, start over with more periodate). From such a result the necessary conditions can be calculated for the actual run, which requires (a) at least twice the periodate rapidly consumed by the solution itself; and (b) at least enough arsenite to take care of the excess periodate. When these conditions are fulfilled, moderate excesses of either reagent cause no trouble.

(11) This procedure guards against loss of acetaldehyde and also facilitates introduction of the reagent against back pressure from the following tubes. (These determinations can, of course, be run in series, in which case a tube of saturated bicarbonate solution should be introduced between each pair of determinations.) It also guards against contamination by acetaldehyde or acetone in the laboratory atmosphere.

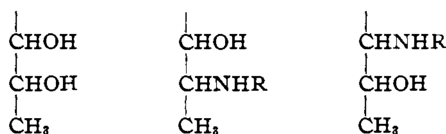
III are mixed and titrated according to the established procedure[12,13] for acetaldehyde formed in the determination of lactic acid, except for the fact that we have used 0.02 *N* iodine solutions where the authors cited used 0.002 *N* solutions.

One cc. of 0.02 *N* iodine solution equals 1.64 mg. of methylpentose. Since it is well established[12,13] that 0.002 *N* iodine can be used for such titrations, it is almost obvious that a ten-fold reduction in the size of sample could, if necessary, be accomplished.

If "other carbohydrates" are to be estimated, the solution remaining in tube I after "aeration" should be treated exactly as for the determination of serine.[5]

## Discussion

While the method described for methylpentoses seems to offer extraordinary promise for the determination of these, it is really not quite so specific as may have *appeared*, and a brief analysis of its scope seems useful for those who think to use it in certain cases. Essentially, it should be a reaction for those substances which contain the groupings




(R = H or a substituent other than acyl)

These include, for instance, such substances as the 2,6-desoxyhexose derivatives digitoxose and cymarose. They include also propylene or 2,3-butylene glycol and, of course, threonine, in so far as this is not in organic combination. They also include *any derivatives* of methylpentoses in which only the 1, 2, or 3 carbons are involved.

Such facts must be considered from time to time, either as the bases for new methods, or as a suggestion of interferences which, with a little ingenuity, may be avoided.

With one exception, we shall probably not follow these possibilities further. But we observe that, under the conditions which are customary in the hydrolysis of polysaccharides, proteins may in certain cases undergo hydrolysis to a rather important extent, and that accordingly, in preparations which contain small amounts of methylpentoses (or the corresponding acids) in polysaccharide form, together with considerable amounts of proteins, the distinction between methylpentose and threonine might be not only interesting, but difficult.

As we have already reported that acyl derivatives of serine do not react with periodic acid, we are now investigating whether a preliminary acylation of the hydrolyzate—probably with acetic anhydride in water solution of suitable *p*H—will not permit a sufficiently sharp distinction between the acetaldehyde (and/or formaldehyde) derived from polysaccharide or protein sources. This project is already moderately successful.

## Summary

A new type of method for the determination of methylpentoses has been described, based on the fact that these are the only sugars which form acetaldehyde when treated with periodic acid. It is believed that this method is outstandingly superior to those previously available.

A procedure is also suggested by which "other carbohydrates" without decision as to their nature, may be determined in the same solution.

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(12) S. W. Clausen, *J. Biol. Chem.*, **52**, 263 (1922).

(13) H. C. Troy and P. F. Sharp, *Cornell Univ. Agr. Expt. Sta. Mem.*, **179** (1935).