DETERMINATION OF THE ISOTOPE DISTRIBUTION IN CARBON LABELLED URIC ACIDS*

by

HANS BRANDENBERGER**

Theodor Kocher-Institute, University of Bern, Bern (Switzerland)

In order to separate for isotopic analysis the atoms of the uric acid carbon chain 4-5-6, position 6 can easily be split off first as CO_2, by oxidation to allantoin with $KMnO_4$, MnO_2 or PbO_2, or to oxonic acid with H_2O_2. The isotopic concentration of this CO_2 has usually been taken as purely that of uric acid position 6[1]. Proof however is lacking that the CO_2 so obtained is not contaminated by carbon from other positions in the molecule. Therefore, synthetically labelled 2-^{14}C-, 4-^{14}C-, 5-^{14}C-, and 8-^{14}C-urid acids were degraded to allantoin (I) and CO_2, and also to oxonic acid (II) and CO_2, in order to determinate the magnitude of such contamination, using the different methods of oxidation. Table I shows that permanganate oxidation to allantoin yields CO_2 amounting to over 98% from position 6. The CO_2 from hydrogen peroxide degradation also carries negligible amounts of radioactivity from positions 2, 4 and 8. In this case, however, a further breakdown of oxonic acid at the carboxyl group (from the former uric acid position 5) occurs, which causes at least a 4% dilution of the CO_2 from position 6, even under very mild conditions. For accurate determination of the isotope concentration in position 6, therefore, the oxidation to allantoin, which has been generally employed in the past, is a preferred procedure. Both degradations, however, will give the same accurate value, if the isotope concentration in position 6 is determined by difference, that is by subtracting the isotope value of the crystalline allantoin or oxonic acid from that of the uric acid sample.

TABLE I

CONTAMINATION OF CO_2 FROM POSITION 6 OF URIC ACID WITH RADIOACTIVITY FROM OTHER POSITIONS

	Contamination from position		
	2 8	*4*	*5*
$KMnO_4$-oxidation to allantoin:	< 1%	< 0.5%	< 0.5%
H_2O_2-oxidation to oxonic acid:			
a. 3% H_2O_2, 30 h	< 1%	< 0.5%	4%
b. 4% H_2O_2, 40 h	—	—	6%

For the separation of uric acid carbons 4 and 5, BUCHANAN *et al.*[1] degraded allantoin (I) from uric acid into glyoxylic acid (IV) and urea. The first was isolated

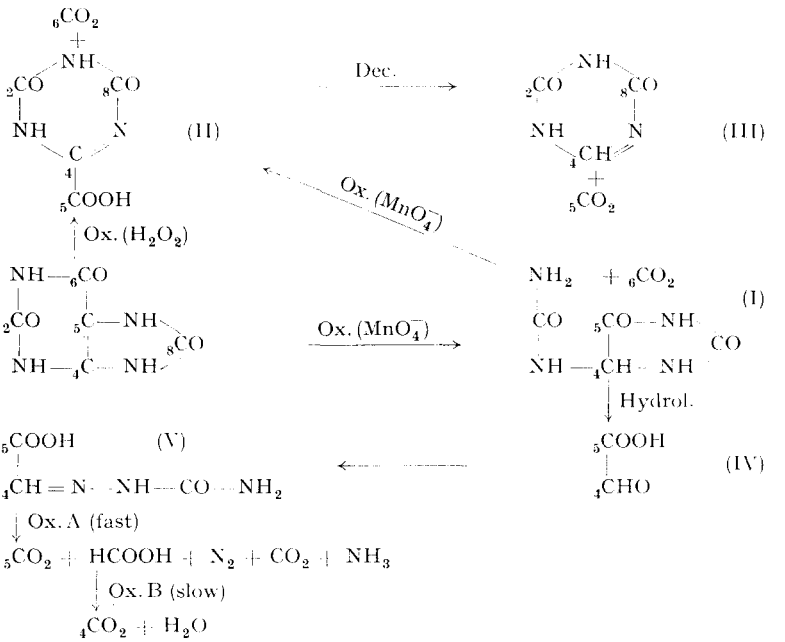
* Communication IV in the serie "Isotope experiments on the degradation of uric acid".

** Present address: Laboratoires Nestlé, Vevey, Suisse.

References p. 522.

as the semicarbazone (V) and converted to CO_2 by permanganate oxidation (Scheme I). The separation of the 2 carbon atoms is based on the fact that the oxidation step A proceeds much more rapidly than reaction B. The CO_2 collected during the first 7 minutes of the reaction was used for evaluation of the isotope concentration in position 5. The CO_2 collected during the next 4 hours, originating mainly from the aldehyde carbon of glyoxylic acid, gave the isotope value of position 4 of uric acid. This method, which has been very useful in the studies of uric acid biosynthesis, has the disadvantages of being inaccurate and requiring at least 80 mg of material.

SCHEME I



Our recent studies[2,4] on the oxidation of uric acid to oxonic acid (II) showed that this compound contains the uric acid position 5 in a carboxyl group which decarboxylates readily upon acidification yielding allantoxaidine (III). The CO_2 split off contains no detectable carbon from other positions and gives therefore an accurate measure of the isotope concentration in position 5 (Table II). This separation of carbon atoms 4 and 5 can be carried out easily with the oxonic acid obtained from as little as 17 mg of uric acid.

TABLE II

CONTAMINATION OF CO_2 FROM POSITION 5 OF URIC ACID WITH RADIOACTIVITY FROM OTHER POSITIONS DURING DECARBOXYLATION OF ONCE RECRYSTALLIZED OXONIC ACID

Contamination from position	2	8	4
% radioactivity in			
experiment 1	0.04	0.00	0.00
experiment 2	0.04		0.75

References p. 522.

In a previous paper[3] a simple indirect method for determining the complete isotope distribution in ^{15}N-labelled uric acids was described, and it was also stated that a similar method could be applied for analysis of carbon labelled uric acids. The specific activities of the five carbons represent five unknowns, which are determined with the help of five independent equations. Each equation requires a determination of the radioactivity of uric acid itself or of one of its degradation products. These compounds (including the uric acid) must contain either a different number of carbon atoms or carbon atoms from different uric acid positions. Only degradation products should be used which can be obtained in a pure state by means of a clearly defined reaction. This will exclude any danger of contamination by the degradation method used; the error of the method, therefore, will be reduced to the errors inherent in the radioactivity determinations.

In an earlier example illustrating the writers method[3], the compounds uric acid, alloxan, oxonic acid, allantoxaidine (or CO_2 from decarboxylation of oxonic acid), and glycine were used to obtain values for the equations. The specific activity of glycine was expressed as a mean of the activities of the positions 4 and 5 of uric acid. If glycine is prepared from uric acid after alkaline oxidation by way of allantoin and hydantoin, this is correct, because such glycine will only contain the former uric acid carbons 4 and 5, the first in the methylene, the second in the carboxyl group[5]. However, if glycine is prepared by direct acid hydrolysis of uric acid, the same equation will not be valid. DALGLIESCH AND NEUBERGER[5] recently found that in the latter case the α-carbon of glycine originates from the former position 5, the carboxyl carbon in equal quantities from positions 4 and 6 of uric acid. Equation A, therefore, should only be used if glycine is prepared by way of allantoin, and should be replaced by equation B, if a direct acid hydrolysis is employed.

TABLE III

CALCULATION OF THE ^{14}C-DISTRIBUTION IN THE FIVE URIC ACID CARBONS BY THE INDIRECT METHOD, USING URIC ACID, ALLOXAN, OXONIC ACID, CO_2 FROM DECARBOXYLATION OF OXONIC ACID, AND GLYCINE FOR RADIOACTIVITY DETERMINATIONS

	A. Glycine by way of allantoin and hydantoin:	B. Glycine by direct acid hydrolysis of uric acid:
I.	$(2)+(4)+(5)+(6)+(8) = (UA)$	$(2)+(4)+(5)+(6)+(8) = (UA)$
II.	$(2)+(4)+(5)+(6) = (Ax)$	$(2)+(4)+(5)+(6) = (Ax)$
III.	$(2)+(4)+(5)+(8) = (OA)$	$(2)+(4)+(5)+(8) = (OA)$
IV.	$(5) = (CO_2)$	$(5) = (CO_2)$
V.	A. $(4)+(5) = (Gl)$	B. $(4)/2+(5)+(6)/2 = (Gl)$
VI.	$(2) = (OA)+(Ax)-(UA)-(Gl)$	$(2) = (Ax)+(CO_2)-2(Gl)$
VII.	$(4) = (Gl)-(CO_2)$	$(4) = (OA)+2(Gl)-(UA)-2(CO_2)$
VIII.	$(6) = (UA)-(OA)$	$(6) = (UA)-(OA)$
IX.	$(8) = (UA)-(Ax)$	$(8) = (UA)-(Ax)$

Symbols: (2), (4), (5), (6), (8) denote the specific molar activities of the carbon atoms in position 2, 4, 5, 6 and 8 respectively, (UA), (Ax), (OA), (CO_2), (Gl) the specific molar activities of uric acid, alloxan, oxonic acid, CO_2 from decarboxylation of oxonic acid and glycine respectively.

Table III shows the differences in the calculation of the complete distribution of the carbon isotope, caused by a change in the glycine degradation procedure. Such a change does not affect the determination of the N-isotope distribution by our

References p. 522.

indirect method, because the glycine nitrogen results from position 7 of uric acid in both cases.

Instead of the degradation products used for establishing the equations of the Table III example, any uric acid derivative can serve for isotope analysis, as long as it meets the algebraic requirements earlier stipulated, and if its carbon atoms can be traced back to the uric acid positions from which they originate.

MATERIALS AND METHODS

The preparation of 2-^{14}C-, 4-^{14}C-, 6-^{14}C-, and 8-^{14}C-uric acids has been described in an earlier communication[3]. 5-^{14}C-Uric acid was kindly donated by Prof. P. P. COHEN and Dr. E. S. CANELLAKIS, Department of Physiological Chemistry, University of Wisconsin, Madison, Wis., U.S.A.

The degradations of the ^{14}C-uric acids to allantoin (I) and to oxonic acid (II) and allantoxaidine (III) were carried out as previously described[3,4]. The CO_2 split off was collected as $BaCO_3$ with the help of an aeration apparatus[3], the radioactivity measured using a Geiger-Müller counter with mica window, and converted to activities at zero thickness. At least two plates were made from each sample.

SUMMARY

1. Methods for the separation for isotopic analysis of the atoms of the uric acid carbon chain 4–5–6 have been investigated in order to determine the magnitude of contamination by carbon from other positions in the molecule.

2. The differences in the calculation of the complete isotope distribution in carbon-labelled uric acids, due to the choice of degradation procedure of uric acid to glycine (direct acid hydrolysis or alkaline hydrolysis after degradation to allantoin and hydantoin), are discussed.

RÉSUMÉ

1. L'éxactitude des méthodes de séparation pour l'analyse isotopique des atomes de carbone numéro 4, 5 et 6 de l'acide urique a été éxaminée.

2. Les résultats des calculs indirects de la répartition complète des isotopes de carbone varient selon le choix des méthodes de dégradation de l'acide urique en glycine (par hydrolyse directe en milieu acide ou en milieu alcalin après dégradation en allantoine et hydantoine).

ZUSAMMENFASSUNG

1. Methoden für die Trennung der Harnsäure-Kohlenstoffatome 4, 5 und 6 für Isotopen-Analyse wurden auf ihre Genauigkeit geprüft.

2. In einem indirekten Verfahren für die Berechnung der vollständigen C-Isotopenverteilung wird hingewiesen auf Unterschiede, die bedingt sind durch die Wahl der Abbaumethode von Harnsäure zu Glycin (direkte saure Hydrolyse oder alkalische Hydrolyse nach Abbau zu Allantoin und Hydantoin).

REFERENCES

1 J. M. BUCHANAN, J. C. SONNE AND A. M. DELLUVA, *J. Biol. Chem.*, 173 (1948) 81.
2 H. BRANDENBERGER, *Helv. Chim. Acta*, 37 (1954) 641; *Chimia*, 7 (1953) 233.
3 H. BRANDENBERGER, *Biochim. Biophys. Acta*, 15 (1954) 108.
4 H. BRANDENBERGER AND R. H. BRANDENBERGER, *Helv. Chim. Acta*, 37 (1954) 2207; *Chimia*, 8 (1954) 262.
5 C. E. DALGLIESH AND A. NEUBERGER, *J. Chem. Soc.*, (1954) 3407.

Received May 13th, 1955