3. H. J. Ranks and D. W. Cameron, Australian J. Chem., 24, 2427 (1971).
4. S. E. Drewes, Phytochem., 10, 2837 (1971).
5. N. A. Tyukavkina, A. S. Gromova, V. I. Lutskii, and V. K. Voronov, Khim. Prirodn. Soedin., 600 (1972).
6. N. A. Tyukavkina, V. I. Lutskii, A. S. Gromova, and N. M. Borodina, in: Phenolic Compounds and Their Physiological Properties [in Russian], Alma-Ata (1973), p. 150.
7. A. S. Gromova, V. I. Lutskii, and N. A. Tyukavkina, Khim. Prirodn. Soedin., 778 (1974).
8. A. S. Gromova, N. A. Tyukavkina, V. I. Lutskii, G. A. Kalabin, and D. F. Kushnarev, Khim. Prirodn. Soedin., 677 (1975).
9. M. W. Dietrich, J. S. Nash, and R. E. Keller, Anal. Chem., 38, 1479 (1966).
10. T. J. Mabry, K. R. Markham, and M. B. Thomas, The Systematic Identification of Flavonoids, Springer-Verlag, New York (1970).
11. H. Gusten and M. Salzwedel, Tetrahedron, 23, 173 (1967).
12. High-Resolution NMR Spectra Catalogue, Varian Associates, 1, No. 305, 306 (1962).
13. T. A. Wittstruck and E. N. Trachtenberg, J. Am. Chem. Soc., 89, 3803 (1967).
14. B. Gurudata, J. B. Stothers, and J. D. Talman, Canad. J. Chem., 45, 731 (1967).
15. I. R. Ader and L. Phillips, J. Chem. Soc., Perkin II, 1975, 1979, 1988, (1972).
16. E. W. Garbisch, J. Chem. Education, 45, a) 311; b) 402, c) 480 (1968).
17. T. M. Tackman and S. Sternhell, Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry, Pergamon Press, Oxford (1969), p. 202.
18. M. Zanger, Organic Mag. Resonance, 4, 1 (1972).
19. W. Brugel, Nuclear Magnetic Resonance Spectra and Chemical Structure, Vol. 1, Academic Press (1967), p. 15.
20. J. Ronayne and D. H. W. Williams, Annual Review of NMR Spectroscopy, 2, 83 (1969).
21. V. J. Kowalewski, Progress in Nuclear Magnetic Spectroscopy, 5, 1 (1969).
22. J. H. Noggle and R. E. Schirmer, Nuclear Overhauser Effect, Academic Press (1971).

DETERMINATION OF URONIC ACIDS IN NATURAL MATERIALS BY THE DECARBOXYLATION METHOD

L. I. Glebko, G. Ya. Il'chenko, and L. P. Kosheleva

UDC 547.917+543.854.7

At the present time, a discussion of the question of the quantitative determination of uronic acids (UAs) in various natural materials is being carried on. A large number of publications has been devoted to improving the decarboxylation method [1]. This method has been fairly well studied in relation to the validity of the results obtained and possible sources of error [2-4], but characteristics of it that are not unimportant in practical work, such as the sensitivity and speed of analysis and the apparatus for the process, require improvement.

An attempt to use this method and also a comparative study of various decarboxylating agents and of the conditions and methods for recording the CO_2 have enabled us to recommend what is, in our opinion, the most promising variant.

The procedure is based on a combination of the decarboxylation reaction of a UA with 57% hydriodic acid (HI) with the direct titration of the CO_2 in nonaqueous solvents [5, 6]. The CO_2 liberated was absorbed by a solution of monoethanolamine (MEA) in dimethylformamide (DMF) and was titrated with a benzene–methanol solution of sodium methoxide.

Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Scientific Center of the Academy of Sciences of the USSR, Vladivostok. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 10–13, January–February, 1976. Original article submitted June 10, 1975.

TABLE 1. Determination of Uronic Acids in Various Polysaccharides by Decarboxylation with 57% HI and with 19% HCl

Substance	Uronic acid (%), found with the aid of			
	57% HI		19% HCl	
D-Glucuronolactone	99,76	100,39	98,00	100.35
D-Galacturonic acid monohydrate	91,45	90,68	90,42	92,55
D-Glucuronic acid	97,46	98,45	98,07	101,04
Alginic acid	78,40	77,93	77,85	78,13
Zosterin*	56,73	57,38	55,90	56,96
Polyuronide from zosterin*	96,72	97,16	96,90	97,80
Citrus pectin	39,58	39,44	40,08	39,92
Sangassan*	24,90	24,86	25,70	24,76
Chondroitin sulfate	23,60	23,76	22,10	23,50
Hyaluronic acid	35,10	35,29	34,70	35,54
Mean value ± mean square deviation at n = 10	64,45±0,40		64,51±1,20	
Mean square deviation, %	0,62		1,86	

*The seaweed polysaccharides were kindly given to us by Dr. Yu. S. Ovodov, and the other materials were commercial preparations.

The advantage of hydriodic acid over other decarboxylating agents such as 12% and 19% HCl consists in a shortening of the reaction time to 30-35 min which, in its turn, decreases the figures obtained in the blank experiments and their fluctuations – factors which limit the sensitivity of any method. As a consequence of the shortening of the time of contact of nonuronic materials with strong mineral acid, the errors due to them are brought to a minimum; the production of well-defined kinetic curves facilitate the recording of the end of the reaction.

The use of nonaqueous titration also has a number of advantages in comparison with indirect determinations of CO_2 [2], IR spectrometry [4], and conductometry [7]. The direct recording of the CO_2 increases the sensitivity of the determination and makes it possible to follow the course of the decarboxylation reaction with time by means of simple apparatus.

The proposed variant of the method ensures the reliable determination of UAs in the range of concentrations from 2 to 100 μM. The lowest determinable concentration is limited by the sensitivity of the method, which, according to a calculation by the 3σ criterion [8], is 1.5 μM of UA, and the upper limit is determined by the precipitation of a large amount of salts, interfering with titration.

We have tested the recommended procedure on polysaccharides of various compositions and structures. The results of determinations of UAs obtained by the use of this variant of the method and with 19% HCl proved to be comparable (Table 1).

EXPERIMENTAL

Reagents. Sodium methoxide solution (0.02 N): 0.46 g of metallic sodium was dissolved in 25 ml of dry methanol in the reservoir of a microburette with 0.01-ml graduations, and the volume was made up to one liter with dry benzene. The titrant was standardized and stored as described in the literature [9]; 1 ml of 0.02 N sodium methoxide solution is equivalent to 0.8802 mg of CO_2. Indicator: 0.1% of Thymol Blue in DMF. MEA: a preparation once-redistilled under vacuum. The 57% HI was prepared and purified as described by Steyermark [10]. The CO_2 liberated in the reaction was freed from volatile impurities interfering with titration (H_2O, HI, I_2, CH_3I, H_2S, etc.) by means of a purification tube (1.2 × 17 cm) filled with a layer (8 cm) of type KSK silica gel with a grain size of 0.5-1.0 mm impregnated with a solution of silver nitrate (5% of $AgNO_3$ on the weight of the silica gel) and activated at 120-130°C for 5 h, and with a layer (5 cm) of pure silica gel. The layers were fixed by glass wool or by treated asbestos. One filling of the tube was sufficient for 40 determinations.

Method. A weighed sample of the material under investigation was placed in a 5-ml reaction flask fitted with a feed tube and with an efficient reflux condenser, and it was covered with 1 ml of 57% HI. The condenser was connected with purifying and gas-outlet tubes, the latter extending to the bottom of an absorption vessel consisting of a test tube (0.8 × 15 cm) with a widening in its central part. The whole system was purged with

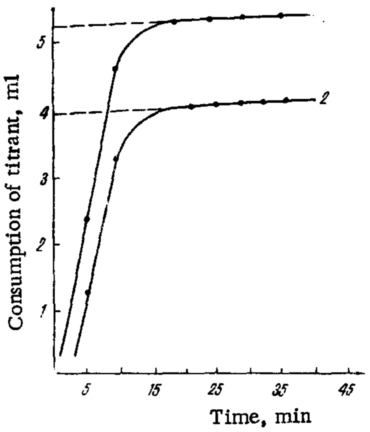

Fig. 1. Graph of the dependence of the consumption of titrant on the time of decarboxylation: 1) galacturonic acid; 2) zosterin – a seaweed pectin.

dry pure argon for 10-15 min, after which 5 ml of DMF, 0.25 ml of MEA, and 1-2 drops of indicator were added to the vessel. A constant flow of argon of 7-8 ml/min was established, and the absorbing solution was neutralized by the titrant to a blue color persisting for 5 min. The reaction mixture was heated in the oil bath to the boil, the CO_2 formed was titrated after predetermined equal intervals of time with the solution of the titrant, and a graph of the consumption of titrant as a function of the time of the reaction was plotted. The determination was complete when the titration curve became almost horizontal. The amount of titrant equivalent to the amount of UA was found from the tangent to the titration curve in its almost horizontal section (Fig. 1). A blank experiment was performed similarly.

SUMMARY

A preferred variant of the decarboxylation method for the determination of uronic acids is proposed which uses 57% hydriodic acid and the direct nonaqueous titration of the CO_2 formed. The range of concentrations of uronic acids that can be determined is from 2 to 100 μM. The sensitivity of the method is 1.5 μM, its reproducibility 0.6%, and the time of one determination 40-50 min. The proposed variant of the method is applicable to the study of various polysaccharides and other carbohydrate-containing biopolymers.

LITERATURE CITED

1. K. V. Lefevre and B. Tollens, Ber., 40, 4514 (1907).
2. D. M. W. Anderson, Talanta, 2, 73 (1959).
3. D. M. W. Anderson and S. Garbutt, Talanta, 8, 505 (1961).
4. D. M. W. Anderson, S. Garbutt, and S. S. H. Zaidi, Anal. Chem. Acta, 29, 39 (1963).
5. J. A. Grant, J. A. Hunter, and W. H. S. Massie, Analyst, 88, No. 1043, 134 (1963).
6. P. Braid, J. A. Hunter, W. H. S. Massie, J. D. Nicholson, and B. E. Pearce, Analyst, 91, No. 1084, 439 (1966).
7. M. Bylund and A. Donetzhuber, Svensk. Papperstidn., 71, No. 15, 505 (1968).
8. R. Kaiser, Z. Anal. Chem., 216, No. 1, 80 (1966).
9. A. P. Kreshkov, Principles of Analytical Chemistry [in Russian], Vol. 2, Moscow (1965), pp. 156-160.
10. A. Steyermark, Quantitative Organic Microanalysis, Blakiston (1951), p. 298.