[CONTRIBUTION FROM THE DIVISION OF APPLIED BIOLOGY, NATIONAL RESEARCH LABORATORIES]

Constitution of Two Aldobiouronic Acids from Wheat Bran Hemicellulose[1,2]

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The acidic hemicellulose of wheat bran, on acid hydrolysis, yielded neutral sugars and a uronic acid fraction. Chromatographic separations of the latter yielded three components of which aldobiouronic acids II and III were examined in detail. Reduction of their methyl glycoside methyl esters with lithium aluminum hydride followed by hydrolysis yielded D-glucose and D-xylose from II and 4-*O*-methyl-D-glucose and D-xylose from III. Methylation of II and III followed by lithium aluminum hydride reduction and hydrolysis, yielded, in both cases, 3,4-di-*O*-methyl-D-xylose and 2,3,4-tri-*O*-methyl-D-glucose. This evidence together with periodate oxidation on the neutral disaccharides obtained by reduction of II and III showed that II was 2-*O*-(α-D-glucopyranosyluronic acid)-D-xylose and III was 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose.

Introduction

Previous investigation of the hemicellulose of wheat bran showed that the main component was a highly branched araboxylan containing 9% uronic acid.[3] Since various fractionation procedures did not yield distinct and separate fractions, it was concluded that the product was reasonably homogeneous. The present paper deals with the constitution of two of the uronic acid components. The uronic acid fraction was removed from a hydrolyzate of the hemicellulose by an anion exchange resin and was shown by paper chromatography to consist of three components. Of these, fractions II and III, whose acid equivalents showed them to be aldobiouronic acids, were studied in detail. Chromatographic examination of a hydrolyzate of II indicated the presence of xylose and glucuronic acid; similar examination of III showed xylose and a monomethyl uronic acid component. It was apparent that the severe conditions required for hydrolysis caused considerable destruction of the products. Accordingly, the aldobiouronic acids were converted to their methyl ester methyl glycosides, reduction of which with lithium aluminum hydride yielded the methyl glycosides of the corresponding disaccharides which were now easily hydrolyzed.[4,5] The disaccharide from fraction II yielded D-xylose and D-glucose, that from fraction III giving D-xylose and 4-*O*-methyl-D-glucose. The unsubstituted sugars crystallized and were identified by melting points and rotations; the 4-*O*-methyl-D-glucose yielded a characteristic crystalline phenylosazone.[6,7] The high positive specific rotations of the aldobiouronic acids (+98° for fraction II, +84.4° for fraction III) indicated that the glycosidic bonds in both of them were in the α-configuration.

In the periodate oxidation of the disaccharide methyl glycoside obtained by reduction of aldobiouronic acid II 2.9 moles of periodate was consumed and 1.16 moles of formic acid was produced. These data fitted the requirements for a glucose unit linked glycosidically to a xylopyranoside unit through $C_{(2)}$ or $C_{(4)}$. Similar oxidation of the disaccharide methyl glycoside from aldobiouronic acid III showed that the linkage of the monomethylated uronic acid must have been to $C_{(2)}$ or $C_{(4)}$ of the xylopyranoside unit.

To establish whether the glycosidic linkages were located at $C_{(2)}$ or $C_{(4)}$ of the xylopyranoside units, aldobiouronic acids II and III were fully methylated. Reduction with lithium aluminum hydride yielded the corresponding glycosides of the disaccharide methyl ethers. Methanolysis and hydrolysis of the disaccharides yielded in each case 3,4-di-*O*-methyl-D-xylose and 2,3,4-tri-*O*-methyl-D-glucose which were identified by formation of crystalline derivatives. These results showed that in both II and III, the uronic acid moiety was joined glycosidically to $C_{(2)}$ of the D-xylose residue.

The above evidence proved that II was 2-*O*-(α-D-glucopyranosyluronic acid)-D-xylose and that fraction III was 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose.

Aldobiouronic acids containing D-xylose and D-glucuronic acid have been isolated from a number of naturally occurring polysaccharides. Investigations on hemicelluloses of aspen wood,[8] Scots pine,[9] beechwood,[10] corncobs[11] and flax[12] have shown that when the D-glucuronic acid contains a methyl ether group on $C_{(4)}$ the glycosidic linkage is always at $C_{(2)}$ of the D-xylose unit. No such order exists when the D-glucuronic acid is unsubstituted, it having been found joined to $C_{(2)}$ of the xylose in sapote gum[13] and wheat bran (the present study), to $C_{(3)}$ of the D-xylose in wheat straw,[14,15] pear cell wall,[16] New Zealand flax[17] and sunflower heads[18] and to $C_{(4)}$ of the D-xylose in corncobs.[19]

Experimental

Paper Chromatography.—Chromatographic separations were carried out by the descending method on Whatman No. 1 paper using the following solvents: (A) ethyl acetate:water:acetic acid:formic acid (18:4:3:1), (B) 2-butanone:water (2:1), (C) ethyl acetate:pyridine:water (2:1:2), (D) benzene:ethanol:water (169:47:15) and (E) 1-butanol:

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(2) Issued as N.R.C. No. 3975.

(3) G. A. Adams, *Can. J. Chem.*, **33**, 56 (1955).

(4) M. Abdel Akher and F. Smith, *Nature*, **166**, 1037 (1950).

(5) B. Lythgoe and S. Trippett, *J. Chem. Soc.*, 1983 (1950).

(6) R. Schinle, *Ber.*, **65**, 815 (1932).

(7) F. Smith, *J. Chem. Soc.*, 2646 (1951)

(8) J. K. N. Jones and L. E. Wise, *ibid.*, 2750 (1952).

(9) A. R. N. Gorrod and J. K. N. Jones, *ibid.*, 2522 (1954).

(10) G. O. Aspinall, E. L. Hirst and R. S. Mahomed, *ibid.*, 1734 (1954).

(11) R. L. Whistler, H. E. Conrad and L. Hough, THIS JOURNAL, **76**, 1668 (1954).

(12) J. D. Geerdes and F. Smith, *ibid.*, **77**, 3569 (1955).

(13) E. V. White, *ibid.*, **76**, 4906 (1954).

(14) C. T. Bishop, *Can. J. Chem.*, **31**, 134 (1953).

(15) G. O. Aspinall and R. S. Mahomed, *J. Chem. Soc.*, 1731 (1954).

(16) S. K. Chanda, E. L. Hirst and E. G. V. Percival, *ibid.*, 1240 (1951).

(17) R. J. McIlroy, *ibid.*, 121 (1949).

(18) C. T. Bishop, *Can. J. Chem.*, **33**, 1521 (1955).

(19) R. L. Whistler and D. I. McGilvray, THIS JOURNAL, **77**, 2212 (1955).

ethanol:water:concd. ammonium hydroxide (100:25:122.5:2.5). Aniline oxalate spray[20] was used to detect the sugars and their methylated derivatives. All specific rotations are equilibrium values unless otherwise stated.

Preparation of Hemicellulose.—"Bee-wing" wheat bran[21] (200 g.) previously extracted with benzene:alcohol (2:1), hot water and ammonium oxalate (0.5%) was extracted twice with potassium hydroxide (4%) under nitrogen for 48 hr. The extract was brought to *p*H 7.0 with acetic acid and concentrated at 28° under reduced pressure to a thin sirup. Dialysis against running water in cellophane tubes for 48 hr. rendered the solution acetate free. After concentration to approximately one-half its volume, the solution was poured into 4 volumes of ethanol with vigorous stirring. The precipitated hemicellulose was dried with ethanol and ether to yield a light gray powder (76 g.). Determination of uronic acid carbon dioxide[22] indicated a uronic acid anhydride content of 9.0%.

Hydrolysis of Hemicellulose and Recovery of Uronic Acid Fraction.—The hemicellulose (75 g.) was heated with *N* sulfuric acid (1500 ml.) in a boiling water-bath until the reducing power[23] became constant (10 hr.). The hot solution was brought to *p*H 6.0 by the careful addition of barium hydroxide. After removal of barium sulfate by filtration, the solution and washings were passed through a column of Amberlite IR-120[24] (30 × 150 mm.) to remove cations. The uronic acid components were then absorbed on a column of Amberlite IR-4B[24] (30 × 200 mm.). The column was washed free of sugar (anthrone test) and the acid components were displaced from the resin with *N* sulfuric acid. The eluate was neutralized by barium hydroxide and after filtration of inorganic barium salts, the excess barium ions were removed from the filtrate by Amberlite IR-120. The acidic solution was concentrated at 30° to a thick clear sirup (6.8 g.). Chromatographic examination in solvent A showed the presence of five components with R_x values (movement relative to that of xylose) as follows: 0.06, 0.35, 0.68, 1.38, 1.70. The last two corresponded to values for 4-*O*-methyl-D-glucuronic acid and D-glucurone, respectively; these sugar acids appeared to be present in relatively small amounts.

Separation of Uronic Acid Components.—The mixture of uronic acid fractions (5 g.) was separated on Whatman No. 1 sheets ($18^1/_4$" × $11^1/_4$") using solvent A. Approximately 100 mg. was placed on each sheet. Three fractions were recovered by water elution of the paper, concentration at 30° and drying *in vacuo* over phosphorus pentoxide to constant weight. Fraction I (2.6 g.), R_x 0.06 (solvent A), appeared to be a mixture and was not studied further. Fraction II (1.22 g.), $[\alpha]^{22}D$ +98°, acid equivalent 336 and R_x 0.29 (solvent A), and fraction III (0.68 g.) $[\alpha]^{22}D$ +84.4°, acid equivalent 355 and R_x 0.68 (solvent A), apparently were aldobiouronic acids.

Examination of Aldobiouronic Acids. Fraction II.—The aldobiouronic acid (15 mg.) was hydrolyzed with *N* sulfuric acid in a sealed tube at 110° for 15 hours. Neutralization with barium carbonate followed by chromatographic examination of the filtrate in solvent (A) showed the presence of xylose and a trace of glucuronic acid.

Identification of the constituents of the aldobiouronic acid was made as follows: the aldobiouronic acid (972 mg.) was converted to the methyl ester methyl glycoside by refluxing with 2% methanolic hydrogen chloride (50 ml.) for 8 hr. After removal of acid with silver carbonate and solvent by evaporation a portion of the methyl ester methyl glycoside (431 mg.) was dissolved in dry tetrahydrofuran (45 ml.) and the solution was added dropwise over a period of 1 hr. to a stirred suspension of lithium aluminum hydride (1.2 g.) in tetrahydrofuran (75 ml.). The reaction mixture was heated under gentle reflux for 30 min., cooled and excess lithium aluminum hydride was decomposed by cautious successive additions of ethyl acetate and water. After filtration, concentration and de-ionization with Amberlite IR-4B and IR-120, the glycoside of the neutral disaccharide was recovered as a foamed solid (374 mg.). Hydrolysis with *N* sulfuric acid (20 ml.) by heating at 97° for 10 hr. yielded xylose and glucose as shown by chromatographic examination of the neutralized hydrolyzate in solvent C. The sugars were separated on large sheets of Whatman No. 1 paper with solvent C. Elution of the papers yielded crystalline D-xylose (51 mg.), m.p. and mixed m.p. 144°, $[\alpha]^{25}D$ +18.2° (*c* 0.8% in water) and D-glucose (92 mg.), m.p. and mixed m.p. 145–146°, $[\alpha]^{25}D$ +50.1° (*c* 0.7% in water), phenylosazone, m.p. and mixed m.p. 204°.

Fraction III.—Acid hydrolysis of this fraction produced a sirup which on chromatographic examination in solvent A yielded sugars corresponding to xylose and 4-*O*-methyl-D-glucuronic acid. The aldobiouronic acid (492 mg.) was converted to the methyl ester methyl glycoside (475 mg.). Reduction with lithium aluminum in tetrahydrofuran as described above yielded the glycoside of the corresponding disaccharide (466 mg.). Hydrolysis with *N* sulfuric acid for 10 hr. at 100° gave a mixture of sugars (362 mg.). On a paper chromatogram, two spots of about equal intensity corresponding to known D-xylose and 4-*O*-methyl-D-glucose were detected. The sirup was separated on large sheets of paper using solvent A. Elution with water yielded D-xylose (31 mg.), m.p. and mixed m.p. 144°, $[\alpha]^{25}D$ +18.3° (*c* 0.6% in water) and 4-*O*-methyl-D-glucose (156 mg.), a sirup (*Anal.* calcd. for $C_7H_{14}O_6$: OCH_3, 16.0. Found: OCH_3, 15.6).

The monomethyl glucose (57 mg.) was converted to its phenylosazone by heating with water (2 ml.), acetic acid (0.2 ml.) and phenylhydrazine (0.3 ml.) at 90° for 3 hr. The 4-*O*-methyl-D-glucosazone which formed was recrystallized from ethanol and had m.p. 157° unchanged on admixture with an authentic sample. The X-ray diffraction patterns of the two specimens were identical thus confirming the identification.

Periodate Oxidation.—The disaccharide methyl glycosides of fractions II and III were oxidized with sodium metaperiodate using the Warburg respirometer method.[25] The results were averages of quadruplicate determinations using 2.35 mg. samples. Formic acid production reached a constant value of 1.16 moles per mole for fraction II after 45 hr. In the case of fraction III no formic acid was produced. The periodate consumptions at this time were 2.9 moles and 2.0 moles per mole for fractions II and III, respectively.

Methylation of Aldobiouronic Acids. 2-*O*-(D-Glucopyranosyluronic Acid)-D-xylose.—The aldobiouronic acid (fraction II, 1.11 g.) was dissolved in water (10 ml.), dimethyl sulfate (10 ml.) was added and the methylation carried out by the dropwise addition of 30% sodium hydroxide (30 ml.) over a 6-hr. period. After stirring overnight, the solution no longer reduced Fehling solution. Solid sodium hydroxide (18 g.) was added and then dimethyl sulfate (25 ml.) with vigorous stirring over 8 hr. This latter methylation procedure was repeated once more. The solution was heated at 95° for 1 hr. to decompose excess dimethyl sulfate, acidified with *N* sulfuric acid (congo red indicator) and extracted continuously with chloroform for 20 hr. Removal of the chloroform yielded a yellow sirup (1.07 g.). Three additional methylations with Purdie reagents (silver oxide, 6 g., and methyl iodide, 20 ml.) yielded a clear sirup (0.982 g.), $[\alpha]^{25}D$ +108° (*c* 1.2 chloroform). *Anal.* Calcd. for $C_{18}H_{32}O_{11}$: OCH_3, 51.2. Found: OCH_3, 51.05.

2-*O*-(4-*O*-Methyl-D-glucopyranosyluronic Acid)-D-xylose.—The aldobiouronic acid (fraction III, 0.896 g.) was fully methylated by the methods described above. The product (750 mg.) had an $[\alpha]^{25}D$ +106° (*c* 0.9 in chloroform). *Anal.* Calcd. for $C_{18}H_{32}O_{11}$: OCH_3, 51.2. Found: OCH_3, 50.8.

Reduction and Hydrolysis of Fully Methylated Aldobiouronic Acid. (a) From 2-*O*-(D-Glucopyranosyluronic Acid)-D-xylose (Fraction II).—The fully methylated acid (0.96 g.) obtained above was dissolved in dry ethyl ether (25 ml.) and reduced with lithium aluminum hydride by the method already described to yield 2-*O*-(2,3,4-tri-*O*-methyl-D-glucopyranosyl)-3,4-di-*O*-methyl-D-xylopyranoside as a yellow sirup (0.87 g.). *Anal.* Calcd. for $C_{17}H_{32}O_{11}$: OCH_3, 47.0. Found: OCH_3, 46.4.

A solution of the methylated disaccharide (0.85 g.) in 8% methanolic hydrogen chloride (50 ml.) was refluxed for 12 hr. After the removal of the methanol, hydrolysis was effected by heating at 100° with 0.5 *N* hydrochloric acid (25 ml.) for 10 hr. The acid was removed with silver carbonate and the solution de-ionized with Amberlite IR-120 and IR-

(20) R. H. Horrocks and G. B. Manning, *Lancet*, **256**, 1042 (1949).

(21) Donated by Flour Mills of America, Inc., Rosedale Mill, Kansas City, Kansas.

(22) M. V. Tracey, *Biochem. J.*, **43**, 185 (1948).

(23) M. J. Somogyi, *J. Biol. Chem.*, **160**, 61 (1945).

(24) Amberlite resins are products of the Rohm and Haas Chemical Co., Philadelphia, Pa.

(25) A. S. Perlin, THIS JOURNAL, **76**, 4101 (1954).

4B. Chromatographic examination in solvent E showed two sugars corresponding to 2,3,4-tri-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-D-xylose. The sirupy mixture of methylated sugars obtained in this way was separated on large sheets of filter paper (Whatman No. 1) using solvent E to give 3,4-di-*O*-methyl-D-xylose (0.143 g.) and 2,3,4-tri-*O*-methyl-D-glucose (0.237 g.).

Identification of 3,4-Di-*O*-methyl-D-xylose.—The R_g values (movement relative to that of 2,3,4,6-tetra-*O*-methyl-D-glucose) for this sirupy product using solvents B and D corresponded to those of a known sample of 3,4-di-*O*-methyl-D-xylose. *Anal.* Calcd. for $C_7H_{14}O_5$: OCH_3, 34.8. Found: OCH_3, 34.4. The 3,4-di-*O*-methyl-D-xylose (0.111 g.) in water (2 ml.) was oxidized with excess bromine in the dark for 50 hr. The bromine was removed by aeration, the solution neutralized with silver carbonate and the residual silver removed with hydrogen sulfide. The final sirup was heated at 15 mm. pressure at 90° for 3 hours to lactonize the acid. On seeding with 3,4-di-*O*-methyl-δ-D-xylonolactone, the sirup crystallized. Recrystallization from ether yielded 3,4-di-*O*-methyl-δ-D-xylonolactone,[26] m.p. 67° and $[\alpha]^{25}D$ −22.3° (*c* 1.5 in water). The X-ray diffraction pattern was identical with that of an authentic sample.

Identification of 2,3,4-Tri-*O*-methyl-D-glucose.—The sirupy material (0.237 g.) obtained above had the same R_g values (0.79, 0.53, 0.87) in solvent B, D and E, respectively, as a known sample of 2,3,4-tri-*O*-methyl-D-glucose and showed $[\alpha]^{25}D$ +69° (*c* 1.3 in water); reported value +66.8°.[27] *Anal.* Calcd. for $C_9H_{18}O_6$: OCH_3, 41.9. Found: OCH_3, 41.5. The trimethylglucose (0.139 g.) was oxidized with nitric acid (15 ml.) (d. 1.2) for 3 hr. at 90°. The acid was removed by successive distillation with water, ethanol and methanol. The sirup was heated for 3 hr. at 90° *in vacuo* and converted to the corresponding ester lactone by refluxing for 6 hr. with 2% methanolic hydrogen chloride. The product was recovered in the usual way and distilled. The 2,3,4-tri-*O*-methyl-D-glucosaccharo-1,5-lactone-6-methyl ester[28] (0.081 g.), b.p. (bath temp.) 140–150° (0.01 mm.), crystallized upon nucleation, m.p. 105–106°, $[\alpha]^{22}D$ +108° in methanol (*c* 0.75) changing in 20 hr. to +58°. *Anal.* Calcd. for $C_{10}H_{16}O_7$: OCH_3, 50.0. Found: OCH_3, 49.6.

(b) **From 2-*O*-(4-*O*-Methyl-D-glucopyranosyluronic Acid)-D-xylose (Fraction III).**—The fully methylated acid (0.593 g.) obtained above gave upon reduction with lithium aluminum hydride as already described, methyl 2-*O*-(2,3,4-tri-*O*-methyl-D-glucopyranosyl)-3,4-di-*O*-methyl-D-xylopyranoside (0.523 g.). *Anal.* Calcd. for $C_{17}H_{32}O_{10}$: OCH_3, 47.0. Found: OCH_3, 46.4.

Hydrolysis of this methylated disaccharide and separation of the cleavage products as described above gave (a) 3,4-di-*O*-methyl-D-xylose (76 mg.), identified as 3,4-di-*O*-methyl-δ-D-xylonolactone, m.p. 67°, $[\alpha]^{25}D$ −22.3° in water, and (b) 2,3,4-tri-*O*-methyl-D-glucose (132 mg.) identified chromatographically as 2,3,4-tri-*O*-methyl-D-glucosaccharo-1,5-lactone-6-methyl ester, m.p. 105–106°.

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(26) S. P. James and F. Smith, *J. Chem. Soc.*, 739 (1945).

(27) S. Peat, E. Schlüchterer and M. Stacey, *ibid.*, 581 (1939).

(28) F. Smith, *ibid.*, 1724 (1939).

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[CONTRIBUTION FROM THE ORGANIC CHEMISTRY SECTION, DIVISION OF CHEMISTRY, NATIONAL BUREAU OF STANDARDS]

Sodium Borohydride Reduction of Aldonic Lactones to Glycitols[1]

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Two procedures are presented for the sodium borohydride reduction of aldonic lactones to glycitols in high yield. In the first procedure, the lactone is reduced at low temperature in aqueous solution, in the presence of sufficient buffer to maintain an acid medium during the first part of the reduction. When all of the lactone has been reduced to the sugar stage or beyond, the *p*H is adjusted to approximately 9, and the reaction mixture is allowed to stand overnight. Cation exchange resin, boric acid, carbon dioxide and sodium acid oxalate were found to be satisfactory buffers. The method has been applied to the preparation of C^{14}-labeled glycitols in yields of 90 to 98%. Directions are given for the preparation of D-mannitol-2-C^{14}. In the second procedure, the lactone is treated with borohydride in anhydrous alcohol. Since the ester that may be formed by alcoholysis, like the original lactone, is reducible, the yield of the glycitol is high.

Labeled glycitols are required as intermediates for the synthesis of D-fructose-1,6-C^{14}, D-glyceraldehyde-3-C^{14}, L-sorbose-6-C^{14}, sedoheptulose-1-C^{14} and other substances needed for biological research. Prior to this investigation, labeled aldonic lactones had been converted to glycitols in yields up to 80%[2] by sodium amalgam reduction to the sugar, followed by high-pressure catalytic hydrogenation. Sodium borohydride[3,4] had also been employed to reduce the lactones in one step to either the aldoses or the glycitols in yields of 25 to 67%.[5,6] Because of the greater convenience of reduction by sodium borohydride, efforts have been made to improve the yields in the reduction of lactones to glycitols by this reagent.

In the previously reported reductions, the aldose was obtained by adding the sodium borohydride to the lactone in a solution maintained at *p*H 3 to 4 by the addition of acid; the glycitol by adding the lactone to an excess of the borohydride. In the latter case, the hydride solution, which was strongly alkaline at the beginning of the reaction, had a *p*H of 8 after complete addition of the lactone. Since, in alkaline solution, sodium borohydride is reported to reduce aldoses nearly quantitatively to glycitols,[7] it seemed probable that the lower yields in the reduction of lactones to glycitols might be due to con-

(1) Part of a project on the development of methods for the synthesis of radioactive carbohydrates, sponsored by the Atomic Energy Commission.

(2) H. S. Isbell and J. V. Karabinos, *J. Research Natl. Bur. Standards*, **48**, 438 (1952).

(3) H. I. Schlesinger and H. C. Brown, THIS JOURNAL, **62**, 3429 (1940).

(4) R. F. Nystrom and W. G. Brown, *ibid.*, **69**, 1197, 2548 (1947); S. W. Chaikin and W. G. Brown, *ibid.*, **71**, 122 (1949).

(5) M. L. Wolfrom and H. B. Wood, *ibid.*, **73**, 2933 (1951).

(6) M. L. Wolfrom and K. Anno, *ibid.*, **74**, 5583 (1952).

(7) (a) M. Abdel-Akher, J. K. Hamilton and F. Smith, *ibid.*, **73**, 4691 (1951); (b) B. Lindberg and A. Misiorny, *Svensk Papperstidn*, **55**, 13 (1952); *C. A.*, **46**, 7942 (1952); (c) P. S. Skell and J. G. Crist, *Nature*, **173**, 401 (1954). Reference a reports a negative Fehling test after reduction, and isolation of the glycitol acetates in 70–92% yield; references b and c report a nearly quantitative gasometric technique.