applied. The intrinsic viscosity, $[\eta]$, was obtained by graphic extrapolation of $\eta_{sp.}/c$ *vs.* c to zero concentration.

The use of trade names in this paper does not necessarily constitute endorsement of the products or of the manufacturers.

Acknowledgments.—The authors are indebted to C. H. VanEtten and B. J. Kinnear for the alkoxyl analyses, J. C. Miers for fractionating the starch and analyzing the fractions, and N. C. Schieltz for X-ray examinations.

PEORIA, ILL. RECEIVED DECEMBER 13, 1950

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF SASKATCHEWAN]

Studies on Lignin by Means of Catalytic Hydrogenation of Aspen Wood and Wheat Straw[1,2]

BY J. M. PEPPER, C. J. BROUNSTEIN AND D. A. SHEARER

Thatcher wheat straw and aspen wood meals have been hydrogenated under conditions which effected complete solubilization of the lignin. The natures of the isolated lignin fractions of these two angiosperms were compared by techniques of separation based on varying degrees of acidity and chromatographic adsorption. Ultraviolet absorption spectra of the several lignin fractions indicated only minor differences, all curves being typical of other isolated lignins and basically aromatic. From straw, but not from wood, methanol is produced by hydrogenation in larger amounts than may be accounted for by the loss of methoxyl groups. From the aspen wood three lignin degradation products were isolated, 4-hydroxy-3-methoxyphenylethane, 4-hydroxy-3,5-dimethoxyphenylethane and 2-(4-hydroxy-3,5-dimethoxyphenyl)-ethanol in yields of 2.0, 2.6 and 0.37% (based on the Klason lignin content of the wood), respectively. It is suggested that the isolation of phenylethane derivatives indicates a β–γ linkage in the protolignin which is susceptible to cleavage under alkaline conditions but not so under neutral or mildly acidic conditions from which by a similar hydrogenation phenylpropane derivatives have been obtained previously.

Previous communications[3,4] have shown that the high pressure catalytic hydrogenation of maple wood meal converted the major part of the lignin fraction into chloroform-soluble oils, which retained the high methoxyl content of the protolignin and which gave all the indications of being aromatic in character. Pure compounds, representing lignin degradation products, were isolated and identified. They were all phenolic in nature, but their yield based on the original Klason lignin content was in no case very high. This method of isolation of lignin has advantages in the use of a starting material containing all of the lignin *in situ* and the stabilization, by reduction, of any reactive groups formed during the pressure cook, thereby minimizing possible secondary polymerization.

The application of this isolation technique to Thatcher wheat straw and aspen (*Populus tremuloides*) meals was undertaken for two reasons. Firstly, since these plant products are both representative of the angiosperm class, the chemical nature of the lignin should be basically similar[5] and similar degradation products should be obtained from each and it was thought of interest to determine if this were correct. Secondly, it was undertaken to study the similarity of the lignin fractions from the above two samples which had been maturing for approximately one-hundred-day and forty-year periods, respectively.

Solvent extracted samples of both wheat straw and aspen wood were hydrogenated in a dioxane–water medium (1:1), containing 3.0% sodium hydroxide in the presence of Raney nickel catalyst under an initial pressure of hydrogen of 3000 lb. per sq. in., for a period of two to three hours at 165–175°. The residual pulp and catalyst were separated in each case from the liquid components by filtration and negative Mäule and phloroglucinol tests on the pulps indicated complete lignin removal. The average yield of the pulp, determined after removal of the nickel by acid treatment, represented 40 and 51% of the original straw and wood, respectively. For each raw material triplicate hydrogenations were made. By analyzing separately the two-thirds of each of these runs and the combined one-thirds, the reproducibility of both the hydrogenation and the solvent extraction procedures (see below) was found to be no better than fifteen per cent.

The components of the alkaline solutions were separated by virtue of their varying degrees of acidity and thereby separate fractions were obtained which represented the chloroform-soluble neutral (A), chloroform-soluble (B) and insoluble (C) mildly acidic (phenolic or enolic), chloroform-soluble (D) and insoluble (E) strongly acidic, and chloroform-insoluble, ether-soluble neutral (F) reaction products. The proportions of each along with their methoxyl contents are given in Table I.

The mildly acidic fraction "B" of the hydrogenation products was chosen for more detailed study. An attempt was made to separate it by chromatographic fractionation on activated alumina from a chloroform solution. Successive elutions, each continued until no further material was removed, with chloroform, acetone, methanol and dilute alkali separated B into four distinct portions referred to as B-1, B-2, B-3 and B-4, respectively. The percentage of Fraction B and of the original Klason lignin as well as the OCH_3 content of each of these portions is given in Table II.

(1) This paper represents part of the theses submitted to the Graduate Faculty of the University of Saskatchewan by C. J. Brounstein and D. A. Shearer in partial fulfillment of the requirements for the degrees of Master of Science and Master of Arts, respectively, 1950.

(2) Presented, in part, before the Division of Cellulose Chemistry at the Meeting of the American Chemical Society, Chicago, Illinois, September 3–8, 1950.

(3) C. P. Brewer, L. M. Cooke and H. Hibbert, THIS JOURNAL, **70**, 57 (1948).

(4) J. M. Pepper and H. Hibbert, *ibid.*, **70**, 67 (1948).

(5) R. H. J. Creighton, R. D. Gibbs and H. Hibbert, *ibid.*, **66**, 32 (1944).

TABLE I

SOLVENT SEPARATION OF HYDROGENATED PRODUCTS[a]

Fraction	Wheat straw Recovery[b]	Wheat straw OMe, %	Aspen wood Recovery[b]	Aspen wood OMe, %
A	7.0	18.2	38.5	25.2
B	19.5	22.3	16.9	23.0
C	13.2	13.0	[c]	..
D	13.9	14.6	6.9	15.2
E	5.1	..	2.4	15.2
F	16.3	8.5	13.3	5.7
G[d]	1.2	14.3	0.0	..
Residual pulp		0.21		0.15
Total	76.2		78.0	

[a] Results based on total recovery of the triplicate runs. [b] Percentage of the original Klason lignin content. [c] Voluminous, mainly inorganic precipitate from which no pure organic fraction was obtained. [d] Chloroform extract (soxhlet) of residual pulp and catalyst.

TABLE II

CHROMATOGRAPHIC SEPARATION OF FRACTION B

Fraction	Percentage fraction B Straw	Percentage fraction B Wood	Percentage Klason lignin Straw	Percentage Klason lignin Wood	OMe, % Straw	OMe, % Wood
B-1	54.7	21.6	10.7	3.7	22.7	23.9
B-2	3.2	18.4	0.63	3.1	12.6	21.1
B-3	25.3	19.0	4.9	3.2	10.9	21.1
B-4	2.8	16.4	0.55	2.8	14.4	18.2
Total	86.0	75.4	16.8	12.8		

As a qualitative method of comparing each of the various fractions obtained in the manners previously described the ultraviolet absorption spectra were determined on representative samples. These curves, along with those of three pure lignin degradation products, are shown in Figs. 1, 2 and 3. They indicate a striking similarity between these isolated lignins of wood and straw and compare favorably with spectra obtained previously for isolated lignins

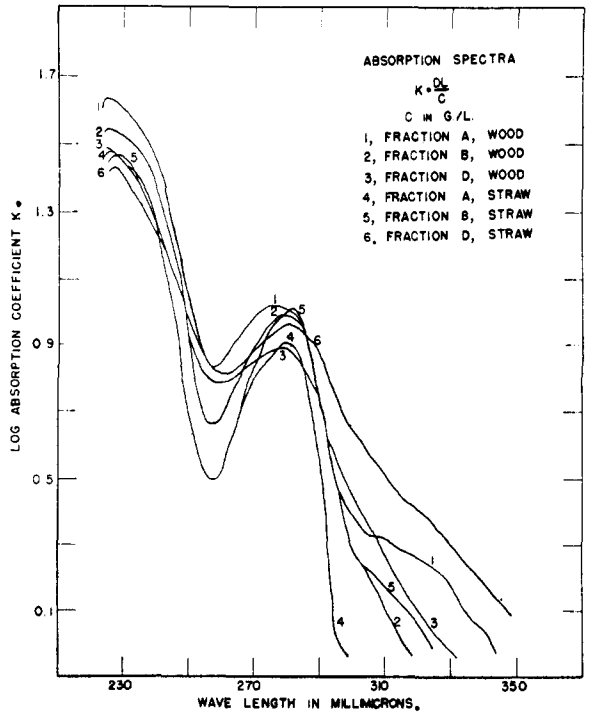


Fig. 1.—Absorption spectra $K = DL/C$, C in g./l.

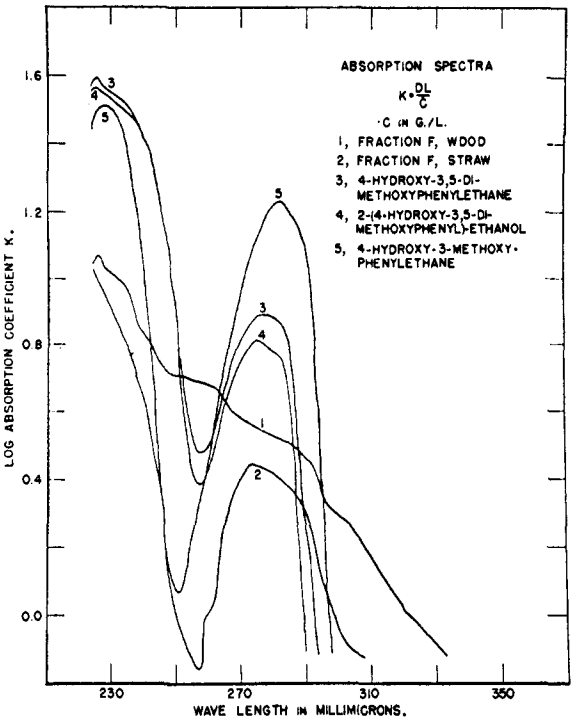


Fig. 2.—Absorption spectra $K = DL/C$, C in g./l.

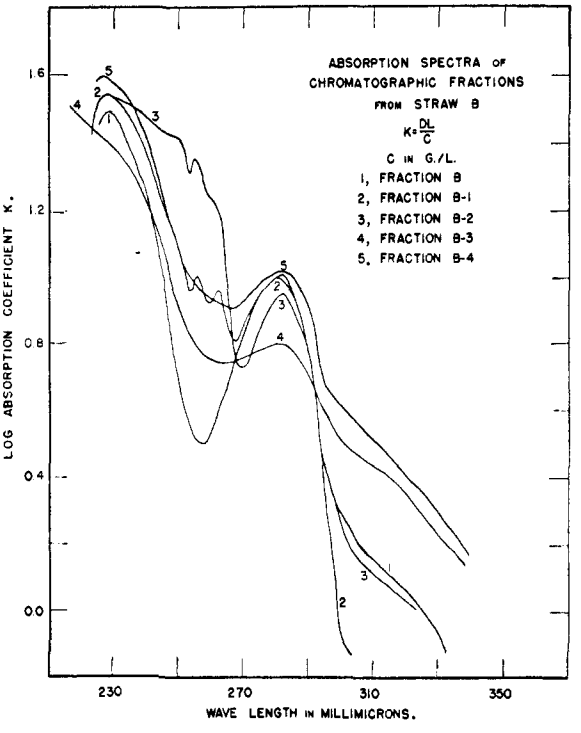


Fig. 3.—Absorption spectra of chromatographic fractions from straw B, $K = DL/C$, C in g./l.

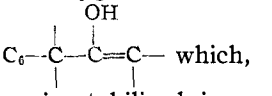
and related compounds.[6,7] The general conclusions drawn by Jones[8] are supported by this work. The absence of any absorption in the 300–350 mμ range is logical due to the highly reducing conditions of the experiment.

(6) R. F. Patterson and H. Hibbert, THIS JOURNAL, **65**, 1869 (1943).

(7) R. E. Glading, *Paper Trade Journal*, **111**, 288 (1940).

(8) E. J. Jones, Jr., *TAPPI*, **32**, No. 7, 311 (1949).

It was of interest to calculate the total recovery of methoxyl in the individual fractions and residual pulp and compare this value with the initial amount in the plant material. On the basis of 100 g. of oven-dried material the original straw had 3.54 g. OMe and the wood contained 5.16 g. OMe. The various recovered products represented only 2.78 g. and 2.77 g. of OMe, respectively. Making the assumption that the balance had been converted into methanol during the course of the hydrogenation, a quantitative estimation of methanol, using the colorimetric procedure of Snell and Biffin[9] was made on representative samples of the reaction products removed immediately after filtration of the bomb contents. Based on 100 g. of starting material the recovery of OMe as methanol represented 2.88 g. for the straw and 2.39 for the wood. A close balance exists in the case of the aspen wood but the large excess methoxyl in the straw experiments would appear to have been formed during the hydrogenation procedure. To test this a sample of straw holocellulose[10] representing 70% of the original straw and having a methoxyl content of 1.37% was treated under similar hydrogenation conditions. Analysis of the reaction filtrate showed the equivalent of 1.48 g. OMe per 100 g. of oven-dried straw. The results then compare only qualitatively but it would appear to indicate that considerable methanol was produced in the hydrogenation from other sources than the lignin fraction.

All attempts to characterize pure compounds from any fraction obtained by the hydrogenation of straw lignin were unsuccessful. In contrast, from the aspen three compounds 4-hydroxy-3-methoxyphenylethane, 4-hydroxy-3,5-dimethoxyphenylethane and 2-(4-hydroxy-3,5-dimethoxyphenyl)-ethanol were isolated in yields of 2.0, 2.6 and 0.37% (based on the Klason lignin content of the wood), respectively. Their recovery parallels the isolation of the same three compounds by the similar treatment of maple wood[4] and adds support to the belief that the lignin fractions of angiosperms are alike chemically. Furthermore, the fact that all three identified compounds are phenylethane derivatives, whereas phenylpropane derivatives were isolated from a similar hydrogenation of maple wood,[3] under mildly acidic conditions, suggests a β–γ linkage in the protolignin of these plants which is susceptible to cleavage under alkaline but not acidic conditions. As has been pointed out previously by Pepper and Hibbert[4] the isolation of 2-(4-hydroxy-3,5-dimethoxyphenyl)-ethanol as a lignin degradation product provides some evidence for a carbon–oxygen linkage through the β-carbon atom of the alkyl side chain. It is not inconceivable that the oxygen functions as part of an enolic system  which, under the alkaline conditions, is stabilized in a form favorable to cleavage of the double bond.

(9) F. D. Snell and F. M. Biffen, "Commercial Methods of Analysis," McGraw-Hill Publishing Co., New York, N. Y., 1944, p. 462.

(10) This sample was obtained through the courtesy of Dr. J. Stone of the National Research Council, Saskatoon, and was the actual material prepared in the experiments described by Adams and Castagne, *Can. J. Res.*, **26**, 325 (1948).

This work indicates that the hydrogenation of both wheat straw and aspen wood gives rise, in each case, to a complex mixture of lignin products but that a general similarity exists between them. There is some evidence to suggest that the lignin of the former is more acidic and less highly methoxylated than that of the much longer-growing wood. Therefore, the rapidly maturing straw would appear to be a suitable source of lignin material for analytical studies especially in any attempts to trace the growth of the lignin fraction by chemical means.

Experimental

Preparation of Plant Material, Wheat Straw.—Mature Thatcher wheat straw meal (20 mesh) was extracted in a soxhlet extractor with a mixture of ethanol and benzene (1:2) for 16 hours, then with ethanol for six hours, thoroughly washed with hot water and finally oven-dried.

Anal. OMe, 3.54; Klason lignin, 24.7.

Aspen Wood.—Wood meal (20-mesh) from the sapwood portion of an approximately forty year old native aspen (*Populus tremuloides*), was extracted continuously with a mixture of ethanol and benzene (4:1) for 48 hours, then with ethanol for 36 hours, thoroughly washed with hot water and finally oven-dried.

Anal. OMe, 5.16; Klason lignin, 16.7.

Hydrogenation of Plant Material.—Three equal portions of solvent extracted, air-dried straw meal were hydrogenated in an Aminco hydrogenator (1410-cc. capacity). A typical run is described as follows: Straw meal (55 g.) was suspended in dioxane–water (1:1) (500 ml.) containing sodium hydroxide (15.5 g.) and hydrogenated over Raney nickel catalyst[11] (40 g. wet with dioxane) at 165–175° for three hours at an initial hydrogen pressure, cold, of 3000 lb./sq. in. In a similar manner three equal portions (55 g. each) of oven-dried wood meal were hydrogenated for 2.5 hours, since preliminary experiments had shown complete lignin removal under these conditions. The maximum pressures recorded were around 4500 lb./sq. in. The average hydrogen absorption was 0.60 and 0.35 mole per 100 g. of oven-dried straw and wood, respectively, determined from cold pressure readings recorded before and after the hydrogenation.

Examination of the Hydrogenation Products. a. Separation of Products According to Degree of Acidity.—The bomb contents were filtered and the pulp and catalyst residue, after washing with aqueous alkali, was extracted in a soxhlet with chloroform (Fraction G). The combined filtrates were then exhaustively extracted with chloroform in the following stages: (a) directly on the bomb filtrate, (b) after acidification with excess carbon dioxide and (c) after complete acidification with hydrochloric acid. Chloroform-insoluble amorphous products separated and were recovered at stages (b) and (c). The residual aqueous solution was continuously extracted with ether for 24 hours. The six fractions thus obtained were referred to as A, B, D, C, E and F in the order mentioned above and except for C and E were oils of varying viscosity. All weights were obtained by drying aliquots to constant weight and are reported only as percentages of the original lignin. Methoxyl analyses were made on these same samples.

b. **Chromatographic Separation of Fractions B.**—Representative samples of Fraction B of both straw and wood products were chromatographed through an alumina column. The alumina, activated by heating to 900° for one hour and screening to 100–150 mesh, was packed into the column by the "slurry" method[12] using chloroform. Elution was carried out successively with chloroform, acetone and finally methanol, each being continued until no further material was removed. A fourth fraction was obtained by extruding the column and extracting with dilute sodium hydroxide.

c. **Ultraviolet Absorption Spectra.**—The absorption spectra of the fractions and compounds listed in Figs. 1, 2 and 3 were determined in purified dioxane with the exception of B-3 (straw) which was insoluble in dioxane and

(11) A. A. Pavlic and H. Adkins, THIS JOURNAL, **68**, 1471 (1946).

(12) A. L. Levy, *Chemistry and Industry*, **64**, 380 (1945).

methanol was therefore used. Concentrations prepared were 0.02–0.04 g. per liter and all measurements were made at minimum slit width with a Beckman DU spectrophotometer using matched 1-cm. quartz cells.

d. Isolation and Identification of Lignin Degradation Products from Aspen Wood (i) Investigation of Fraction A.—Part (8.2 g.) of the "neutral" fraction A obtained by the hydrogenation of aspen wood meal was divided into an ether-insoluble, dark amorphous material (0.43 g.) and an ether-soluble oil (7.7 g.). Of this latter fraction, a portion (4.5 g.) was distilled through a small column (7 in. by 1.6 in. diameter) packed with beads (av. diameter 4 mm.). The distillate (2.03 g.) was thereby divided into eight fractions obtained over a boiling range 75–160° at 1 mm.

4-Hydroxy-3-methoxyphenylethane.—From the fraction boiling 82–87° (1 mm.) (0.56 g.) the *p*-nitrobenzoate derivative was prepared in good yield and after two crystallizations from ethanol–water melted 91.5–93°; mixed m.p. with an authentic sample of 4-hydroxy-3-methoxyphenylethane[13] (m.p. 95.5–96.5°) was 92.5–95°.

Anal. Calcd. for $C_{16}H_{15}O_5N$: OCH_3, 10.3. Found: OCH_3, 10.1.

4-Hydroxy-3,5-dimethoxyphenylethane.—From the fraction boiling 87–111° (1 mm.) (0.70 g.) the *p*-nitrobenzoate derivative was prepared, which, after three crystallizations from ethanol, melted at 150.5–151.5°. A mixed m.p. with an authentic sample[4] from 4-hydroxy-3,5-dimethoxyphenylethane (m.p. 152–153°) was 151–152.5°.

(13) Prepared by the Clemmensen reduction of 4-hydroxy-3-methoxyphenyl methyl ketone.

Anal. Calcd. for $C_{17}H_{17}O_6N$: OCH_3, 18.7. Found: OCH_3, 18.4.

(ii) Investigation of Fraction B-1. 2-(4-Hydroxy-3,5-dimethoxyphenyl)-ethanol.—Part (0.64 g.) of the partially crystalline fraction B-1 was washed with ether (5 ml.). This removed the oily fraction and left a white crystalline product, weight 0.065 g., m.p. 103–109°. Twice crystallized from ether, petroleum ether and ethanol this product melted at 112–113°. A mixed m.p. with an authentic sample[4] of 2-(4-hydroxy-3,5-dimethoxyphenyl)-ethanol (m.p. 113–114°) was 112–113°.

Anal. Calcd. for $C_{10}H_{14}O_4$: OCH_3, 31.3. Found: OCH_3, 30.8.

Acknowledgment.—The authors wish to thank the National Research Council of Canada for the award of a Bursary and a Summer Scholarship to one of them (C. J. B.) and also to thank the Saskatchewan Research Council for both the award of a Fellowship to another (D. A. S.) and other financial assistance. The preparation and extraction of the straw meal was carried out in the Prairie Regional Laboratories of the National Research Council, Saskatoon, for which coöperation acknowledgment is gratefully made.

SASKATOON, SASKATCHEWAN, CANADA

RECEIVED OCTOBER 27, 1950

[CONTRIBUTED FROM THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

On the Utilization of Guanine by the Rat[1]

BY M. EARL BALIS, DENIS H. MARRIAN AND GEORGE BOSWORTH BROWN

The administration of guaninine-8-C^{14} to the Sherman rat has now demonstrated an extremely small utilization of this compound, which escaped detection in previous investigations with N^{15}-labeled guanine.

The initial studies of the metabolism of N^{15}-labeled guanine by Plentl and Schoenheimer[2] led to the conclusion that guanine was not utilized by the rat for the synthesis of the tissue polynucleotides. This was repeated and confirmed in this Laboratory with Sherman strain rats, for both orally[3] and intraperitoneally[4] administered N^{15}-labeled guanine. Subsequent studies with this guanine have shown, however, that it may be utilized to a significant extent by the C57 black mouse.[5]

The guanine used in these experiments[2,3,5] contained N^{15} in both the 1- and 3-nitrogens of the ring and in the 2-amino group. Although there is little degradation *in vivo* of the purine ring,[6] the substituent 2-amino group of guanine is lost in the catabolism to allantoin and contributes appreciable isotopic ammonia to the body-pool. This, in turn, leads to a small incorporation of N^{15} into most of the nitrogenous compounds and this obscures any very small specific incorporation of the intact purine. The availability of guanine-8-C^{14} [7] of high specific activity circumvents this difficulty and it also offers an isotope which can be determined at a greater dilution than could the N^{15}.

The study of guanine-8-C^{14} administered at a level of 0.2 mM/kilo./day for three days to the Sherman strain white rat has now led to the detection of a very small specific incorporation of guanine into the polynucleotide guanine (Table I).[7a] In view of the inactivity of the polynucleotide

TABLE I

UTILIZATION OF GUANINE-8-C^{14} BY THE SHERMAN RAT, 0.2 MM/KG./DAY FOR 3 DAYS

	C.p.m./μM.	Relative specific activity[a] × 10^2
Guanine (injected)	17.6×10^3	100
PNA adenine	<1	0.0
PNA guanine	17	0.1
DNA	<1	0.0
Allantoin	3.2×10^3	18.3
Urea nitrate	2.1	0.01
Urea (as $BaCO_3$)	1.7	0.01

[a] Relative activity of isolated compound and administered guanine.

(1) (a) The authors wish to acknowledge the support of the Atomic Energy Commission, Contract AT(30-1)-910, the National Cancer Institute of the United States Public Health Service and the Nutrition Foundation, Inc. (b) Presented in part at the 119th Meeting, American Chemical Society, Boston, April, 1951.

(2) A. A. Plentl and R. Schoenheimer, *J. Biol. Chem.*, **153**, 203 (1944).

(3) G. B. Brown, P. M. Roll, A. A. Plentl and L. F. Cavalieri, *ibid.*, **172**, 469 (1948).

(4) A. Brandt, P. M. Roll and G. B. Brown, unpublished.

(5) G. B. Brown, A. Bendich, P. M. Roll and K. Sugirua, *Proc. Soc. Exptl. Biol. and Med.*, **72**, 501 (1949).

(6) G. B. Brown, Cold Spring Harbor Symposia on Quantitative Biology, XIII, 43 (1948).

(7) M. E. Balis, G. B. Brown, G. B. Elion, G. H. Hitchings and H. VanderWerff, *J. Biol. Chem.*, **188**, 217 (1951).

(7a) NOTE ADDED IN PROOF.—Recently R. Abrams and J. M. Goldinger, *Arch. Biochem.*, **30**, 261 (1951), have mentioned that they have found guanine to be incorporated into rat intestinal PNA.