that of vitamin B_2, B_{12}, and other known vitamins. Nicotinic acid is one precedent for a substance being known chemically before recognition as a vitamin. Besides vitamin tests, lyxoflavin was desired for tests on the growth of lymphosarcoma transplants in mice, since 6,7-dichloro-9-(1′-D-sorbityl)-isoalloxazine[4] enhanced regression and yet was not an inhibitor of riboflavin.

Our tests show that synthetic lyxoflavin[5] is devoid of riboflavin activity in rats by the standard assay as it should be if it is a distinct vitamin-entity.

Ershoff[6] found that a water-insoluble liver fraction counteracted the growth depressing effect of a casein diet containing thyroid powder. Addition of vitamin B_{12} did not give a growth response in this test.

We have maintained rats on a deficient diet consisting of soybean meal, dextrose, minerals, the known vitamins and 0.5% thyroid powder which enhances development of a deficiency state. After depletion for twenty-eight days, vitamin B_{12} was added to the basal ration. When natural sources of unidentified vitamins were added, a weight gain resulted (Table I).

TABLE I

RAT ASSAY FOR UNIDENTIFIED VITAMINS

Dietary groups	No. male rats	15-day weight gain
Basal	50	64 g.
Defatted liver powder (10%) Viobin Corp.	30	77 g.
Menhaden fish meal (10%)	20	79 g.
Water insoluble liver solids (10%)	10	83 g.

We have found in two assays (Table II) that supplementation with lyxoflavin resulted in weight gains comparable with those observed with liver and fish meal, namely, 20–35% above that of the control groups.

TABLE II

TESTS ON LYXOFLAVIN FOR GROWTH ACTIVITY IN RATS

Groups of rats (9–11 males each)	15-day weight gain, g.
Basal (expt. 1)	64
Plus 150 μg. lyxoflavin daily (expt. 1)	78
Basal (expt. 2)	64
Plus 150 μg. lyxoflavin daily (expt. 2)	88

Thus, it appears that lyxoflavin has growth-promoting or vitamin-like activity in rats. These tests are being extended with rats and with microorganisms, chicks and other animals.

(4) Holly, Peel, Mozingo and Folkers, *ibid.*, **72**, 5416 (1950).
(5) Heyl, Cates, Koniuszy and Folkers, *ibid.*, **73**, in press (1951).
(6) Ershoff, *Proc. Soc. Exp. Biol. Med.*, **73**, 459 (1950).

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THE SEPARATION OF MONOSACCHARIDES BY ION EXCHANGE[1]

Sir:

The biosynthetic preparation of C^{14}-labeled sugars requires a method for the separation of individual monosaccharides from their mixtures. The methods used up to the present time have, in general, been based upon adsorptive chromatography and both columnar and paper partition chromatography.[2] In many cases they are non-quantitative, or difficult, or impossible to apply to preparative isolations, or include undesirable manipulative procedures and chemical conversions.

Since borate ion reacts with sugars to produce negatively charged sugar–borate complexes,[3] it occurred to us that such sugar–borate complexes might be separable by the technique of ion exchange in a fashion similar to that used in separating nucleotides and related compounds.[4]

It was found that fructose, glucose, mannose and galactose, dissolved in weak sodium borate solutions, are quantitatively adsorbed on strong-base anion exchangers. Elution was carried out with dilute sodium borate solutions and the effluent fractions were analyzed for sugar by the quantitative anthrone method of Dreywood[5] as developed by Morris.[6] Identification of the sugars was accomplished by paper chromatography.[7]

A separation of glucose, galactose and fructose on a strong-base anion exchanger is shown in Fig. 1. Recoveries of the individual sugars were essentially quantitative.

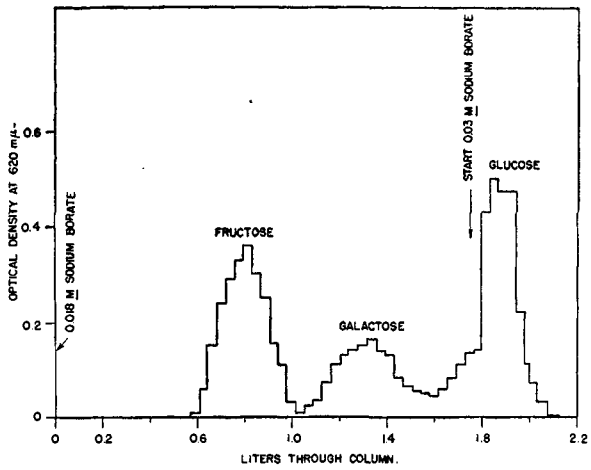

Fig. 1.—Exchanger, 0.85 sq. cm. × 11 cm. Dowex-1, *ca.* 300 mesh, borate form; eluting agent; sodium borate as shown at 1 ml./min.; test material, 10 mg. fructose, 12.5 mg. galactose, 12.5 mg. glucose in 10 ml. of 0.01 *M* sodium borate; recovery, ~99%, based on optical density at 620 mμ.

While a number of variables remain to be explored and the method has not yet been extended to in-

(1) Work performed under Contract Number W-7405-Eng-26 for the Atomic Energy Commission.

(2) S. Udenfriend and M. Gibbs, *Science*, **110**, 708 (1949); W. W. Binkley and M. L. Wolfrom, "Chromatography of Sugars and Related Substances, Sugar Research Foundation, Inc., Scientific Report Series No. 10 (1948); B. W. Lew, M. L. Wolfrom and R. M. Goepp, Jr., THIS JOURNAL, **68**, 1449 (1946); G. R. Noggle and R. A. Bolomey, *Plant Physiol.*, in press.
(3) J. Böeseken, *Advances in Carbohydrate Chem.*, **4**, 189 (1949); H. S. Isbell, J. F. Brewster, N. B. Holt and H. L. Frush, *J. Res. Nat. Bur. Standards*, **40**, 129 (1948); Y. Tuzuki, *Bull. Chem. Soc., Japan*, **16**, 23 (1941).
(4) W. E. Cohn, THIS JOURNAL, **72**, 1471 (1950); E. Volkin, J. X. Khym and W. E. Cohn, *ibid.*, **73**, 1533 (1951).
(5) R. Dreywood, *Ind. Eng. Chem., Anal. Ed.*, **18**, 499 (1946).
(6) D. L. Morris, *Science*, **107**, 254 (1948).
(7) S. M. Partridge, *Biochem. J.*, **42**, 238 (1948).

clude other hexoses, the pentoses, and the disaccharides, it has promise of wide application to many of the separations and analytical problems involved in investigations of sugars and other compounds which are capable of forming charged borate complexes. Further studies are in progress and will be published later.

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DEGRADATION OF TERRAMYCIN

Sir:

This is a preliminary report on the most significant degradative reactions carried out in our laboratory on the new broad spectrum antibiotic, terramycin,[1,2]

Terramycin, $C_{22}H_{24-26}N_2O_9$, is readily degraded by the action of aqueous alkali. On boiling a 20% aqueous sodium hydroxide solution of terramycin, one mole each of ammonia and dimethylamine are evolved within 24 hours. When the hydrolysis is carried out in the presence of zinc, a number of crystalline products can be isolated. The major product, isolated in 50% yield as a white crystalline compound, has been named terracinoic acid (m.p. 232–234°, dec.). *Anal.* Calcd. for $C_{13}H_{12}O_6$: C, 59.09; H, 4.58. Found: C, 59.17, 59.09; H, 4.40, 4.84. Terracinoic acid is a tribasic acid with pK_a values 2.6, 4.7 and 9.1. Among the products isolated in relatively low yield from this reaction mixture is a white crystalline phenolic lactone (m.p. 110–112°). *Anal.* Calcd. for $C_9H_8O_3 \cdot H_2O$: C, 59.33; H, 5.54; H_2O, 9.89. Found: C, 59.32; H, 5.79; H_2O (K.F.), 9.30. Acetic acid and carbon dioxide are also produced in this alkaline degradation.

Salicylic, *m*-hydroxybenzoic and succinic acids have been isolated from a potassium hydroxide fusion of terramycin carried out at 200°.

The carbon skeleton of terramycin is cleaved less readily in acidic media. Terramycin is slowly rearranged by two equivalents of 1 *N* hydrochloric acid at 60° to yield a yellow crystalline hydrochloride (m.p. 198–202°, dec.). *Anal.* Calcd. for $C_{22}H_{24}N_2O_9 \cdot HCl$: C, 53.17; H, 5.07; N, 5.64; Cl, 7.14. Found: C, 53.37; H, 5.33; N, 5.57; Cl, 7.23. This rearrangement product is optically active but has no biological potency. The free base is a stronger acid than terramycin.

More vigorous treatment of terramycin in acid solution results first in removal of dimethylamine and carbon dioxide, and finally in the loss of the second nitrogen function. Among the products of vigorous acid treatment are: (1) a crystalline derivative (m.p. 210–213°, with prior darkening) *Anal.* Calcd. for $C_{19}H_{17}NO_8$: C, 58.91; H, 4.39; N, 3.62. Found: C, 59.11; H, 4.60; N, 3.45; and (2) an air-sensitive nitrogen-free compound (decomposes over a range 215–245° without melting). *Anal.* Calcd. for $C_{15}H_{12}O_6$: C, 62.50; H, 4.20. Found: C, 62.12; H, 4.38.

(1) A. C. Finlay, *et al.*, *Science*, **111**, 85 (1950).

(2) P. P. Regna, I. A. Solomons, A. E. Timreck, K. Murai, K. J. Brunings and W. A. Lazier, THIS JOURNAL, in press.

The dimethylamino group is cleaved readily from terramycin by the action of zinc and glacial acetic acid at room temperature. The remaining carbon skeleton is accounted for by the isolation in good yield of a pale yellow crystalline compound (m.p. 175–180°, dec.). *Anal.* Calcd. for $C_{20}H_{21}NO_8$: C, 59.55; H, 5.25; N, 3.47. Found: C, 59.23; H, 5.41; N, 3.38, 3.59. This compound does not form a hydrochloride and is a stronger acid than terramycin.

Further details of the degradation of terramycin will be published as the work progresses.

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RECEIVED APRIL 6, 1951

BROMINATION OF HECOGENIN ACETATE

Sir:

Current interest in the synthesis of cortisone from 12-ketosapogenins prompts us to report some preliminary results of work with hecogenin. In considering the introduction of an oxygen atom at C-11 by hydrolysis of 11,23-dibromohecogenin acetate (I) we were first concerned with the stability of the halogen in 23-bromohecogenin acetate[1] toward hot alkaline hydrolysis; it was found to be resistant toward such treatment since 23-bromohecogenin was the product. The acetate is unchanged in the presence of boiling pyridine.[1] Further bromination resulted, however, in I, whose structure was assigned on the basis that removal of hydrogen bromide in pyridine gave an α,β-unsaturated ketone, designated as II, whereas mild alkaline hydrolysis removed only one of the bromine atoms with simultaneous hydrolysis of the ester to give III. In view of the stability of the 23-bromoketospirane side chain under the conditions used these reactions place the reactive bromine at C-11. This assignment is supported by similar studies in the bile acid series from which, also, a method is indicated for preparing the 11-keto derivatives by hydrolysis of the halides and rearrangement of the resulting 11-hydroxy-12-ketones in hot alcoholic alkali.[2]

Hydrolysis of 23-bromohecogenin acetate yielded 23-bromohecogenin, m.p. 210° (dec.)[3], $[\alpha]^{26}D$ −3.0° (dioxane). Calcd. for $C_{27}H_{41}O_4Br$: C, 63.64; H, 8.11; Br, 15.68. Found: C, 63.10; H, 8.05; Br, 15.64. Bromination of the acetate in glacial acetic acid at room temperature with a slight excess of bromine gave 11,23-dibromohecogenin acetate (I), m.p. 173° (dec.), $[\alpha]^{26}D$ −21.4° (ethanol). Calcd. for $C_{29}H_{42}O_5Br_2$: C, 55.24; H, 6.72; Br, 25.34. Found: C, 55.52; H, 6.79; Br, 25.37. Treatment of this product with hot pyridine yielded 9,(11)-dehydro-23-bromohecoge-

(1) R. E. Marker, R. B. Wagner, P. R. Ulshafer, E. L. Wittbecker, D. P. J. Goldsmith and C. H. Ruof, THIS JOURNAL, **69**, 2167 (1947).

(2) *Cf.* T. F. Gallagher, *J. Biol. Chem.*, **162**, 539 (1946); T. F. Gallagher and E. Borgstrom, *ibid.*, **164**, 791 (1946).

(3) All melting points were observed at fifty magnifications on the Kofler hot stage and are corrected.