tose it became of interest to learn at what stage of our experiment a partial transformation of the glucose moiety to fructose had taken place. During the working-up procedure of the acid hydrolysate use was made of a strongly basic anion exchange resin, Amberlite IRA-400 (OH form)[1], for neutralization. It seemed logical to assume that the conversion was caused by the catalytic action of the resin in a reaction reminiscent of the base-catalyzed Lobry du Bruyn transformation. This was borne out by the fact that when 25 ml. of a 2% glucose or fructose solution was allowed to stand in contact with 3 g. of the resin, the following analytical data were obtained by combination of the alkaline hypoiodite and the Somogyi methods.

TABLE I

Time, hr.	Starting glucose % Glucose	Starting glucose % Fructose	Starting fructose % Glucose	Starting fructose % Fructose
0	100	..	..	100
24	92.9	7.1	21.1	78.9
48	84.3	15.7	37.1	62.9
120	75.3	24.7	52.3	47.7
168	71.4	28.6	52.7	47.3
212	70.3	29.7	54.3	45.7

The percentages given above refer only to the carbohydrate remaining, since it was found that approximately 30% of the sugars was destroyed into acidic products. Acidic degradation of certain sugars was reported by Phillips and Pollard[2] and by Hulme,[3] who chromatographically detected, though not completely identified, at least five acidic residues.

These results made it desirable to determine what effect the resin has on other sugars. When cellobiose was allowed to remain in contact with the resin for 70 hours, there was detected, in addition to unreacted cellobiose, an unidentified disaccharide as well as a considerable quantity of glucose and fructose. The amount of glucose and fructose increased with time until after 238 hours most of the disaccharides disappeared. Similar results were obtained with maltose where the other disaccharide was assumed to be maltulose. When turanose was allowed to remain in contact with the resin for 46 hours, the only sugars that could be detected chromatographically were glucose and fructose. In all these disaccharide reactions there was an accompanying destruction of the carbohydrate to acidic products analogous to the glucose case. No reaction was observed with sucrose and several unidentified spots were obtained with D-arabinose, one of them presumably representing ribulose.

It also became of interest to learn what reaction, if any, occurred when the resin was in the carbonate rather than the hydroxyl form. Neither maltose nor glucose underwent any conversion after 48 hours. Turanose, however, was converted in considerable amount to glucose with the complete absence of fructose.

When glucose was treated with a weakly basic resin, Amberlite IR-4B,[1] there was detected, after 48 hours, a weak spot of fructose beside glucose on the chromatogram.

(1) Manufactured by Rohm and Haas Co., Philadelphia, Pa.
(2) J. D. Phillips, and A. Pollard, *Nature*, **171**, 41 (1953).
(3) A. C. Hulme, *Nature*, **171**, 610 (1953).

The mechanism of these reactions cannot be postulated on the basis of these preliminary experiments. However, it appears that the presently accepted ene-diol mechanism for the Lobry du Bruyn transformation does not hold in this case, as evidenced by the absence of mannose in the glucose–fructose interconversion.

These results bring to light two important considerations which should be accorded the strictest attention. First, extreme care must be exercised in using such ion exchange resins in conjunction with solutions of reducing carbohydrates. Careful analysis must be performed on the column effluent to determine whether any considerable resin-catalyzed reaction took place. Second, one must consider the use of these anionic resins as catalysts for facile interconversions of carbohydrates. The evident advantage of easy removal of catalyst from the reaction mixture makes such a possibility most attractive.

Investigations along these lines are being continued.

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RECEIVED AUGUST 3, 1953

THE CONVERSION OF L-LYSINE-6-C^{14} TO PIPECOLIC ACID IN THE RAT

Sir:

L-Pipecolic acid recently has been determined to be a constituent of certain plants.[1,2] This amino acid bears a close structural relationship to lysine, and while investigating the metabolism of the latter in rats, we have found evidence that L-lysine-6-C^{14} is converted in significant measure to radioactive pipecolic acid. The method used in making this observation was one which has been utilized successfully in this laboratory for detecting other specific catabolites of radioactive precursors. A solution containing 6.4 mg. of L-lysine-6-C^{14} monohydrochloride (3.50×10^8 disintegrations/min./mMole) and 500 mg. of L-pipecolic acid[3] was injected intraperitoneally into a male Wistar rat which had previously been fasted for 24 hours. The urine was collected for 24 hours, filtered and passed consecutively through columns of the ion exchangers Amberlite IR-4 and IRC-50. The effluent was evaporated to dryness, and the residue was converted to a copper salt by treatment with copper carbonate in 95% ethanol. After treatment of the copper salt with hydrogen sulfide in hydrochloric acid solution, the L-pipecolic acid was recovered as the hydrochloride from an ethanol–acetone mixture. Two recrystallizations yielded approximately 70 mg. of a material which showed only one spot on a ninhydrin treated paper chromatogram (collidine–lutidine–water). The spot corresponded to that obtained with authentic

(1) R. M. Zacharius, J. F. Thompson and F. C. Steward, THIS JOURNAL, **74**, 2949 (1952). See also N. Grobbelaar and F. C. Steward, *ibid.*, **75**, 4341 (1953).
(2) R. I. Morrison, *Biochem. J.*, **53**, 474 (1953).
(3) The authors wish to thank Dr. F. C. Steward of Cornell University for his generous gift of this compound.

L-pipecolic acid and fluoresced cherry red under ultraviolet light as reported by Morrison.[2] An assay of the pipecolic acid hydrochloride showed that it had a specific activity of 1.21×10^5 disintegrations/min./mmole. Part of the pipecolic acid hydrochloride was converted to the hydantoin[4] and this derivative had a specific activity of 1.23×10^5 disintegrations/min./mmole. These observations afford strong evidence that pipecolic acid is a catabolite of L-lysine in the rat.

The high specific activity obtained suggests that pipecolic acid is involved in the conversion of L-lysine to α-aminoadipic acid, a view in keeping with the finding that under similar experimental conditions, L-lysine, via α-aminoadipic acid, yields glutaric acid with a specific activity of 6.45×10^4 disintegrations/min./mmole.[5]

(4) W. Leithe, *Ber.*, **65**, 927 (1932).
(5) M. Rothstein and L. L. Miller, unpublished results.

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RECEIVED JUNE 26, 1953

THE MINOR ALKALOIDS OF *GELSEMIUM SEMPERVIRENS*[1]

Sir:

In the course of our work with gelsemine the isolation of the alkaloids of *Gelsemium sempervirens* Ait. has been reinvestigated. The alkaloidal residue obtained from the combined mother liquors left after removal of all the gelsemine and sempervirine was benzoylated to separate the secondary from the tertiary amines. The neutral fraction, after purification by chromatography, crystallized readily. It was hydrolyzed and the recovered base converted to a perchlorate which on repeated recrystallization from methanol–water was separated into a very sparingly soluble crystalline perchlorate and a readily soluble one. The readily soluble perchlorate yielded alkaloid A, m.p. 171–172°, $[\alpha]^{25}D$ −142° (*c*, 0.945 in $CHCl_3$). *Anal.* Found: C, 66.89, 67.27; H, 7.00, 7.31; N, 7.78; OCH_3, 16.47; NCH_3, 3.96. Calcd. for $C_{20}H_{26}O_4N_2$: C, 67.02; H, 7.31; N, 7.82; 2 OCH_3, 17.30; 1NCH_3, 4.18. The base which contains one C-methyl and one active hydrogen (Zerewitinow) forms a neutral benzoyl derivative, m.p. 235–236°, $[\alpha]^{25}D$ −107° (*c*, 0.97 in $CHCl_3$). *Anal.* Found: C, 70.02; H, 6.50; N, 6.21. Calcd. for $C_{27}H_{30}O_5N_2$: C, 70.11; H, 6.54; N, 6.06. These properties are in agreement with those reported by Chou[2] and by Forsyth, Marrian and Stevens[3] for gelsemicine. Furthermore, the ultraviolet and infrared absorption spectra of alkaloid A were identical with the corresponding spectra determined on a sample of Chou's gelsemicine.[4] In admixture with Chou's gelsemicine (m.p. 164–167°), alkaloid A melted at 168–170°. Alkaloid A, therefore, is identical with gelsemicine.

(1) Issued as N.R.C. Bull. No. 0000.
(2) T. Q. Chou, *Chinese J. Physiol.*, **5**, 131 (1931).
(3) W. G. C. Forsyth, S. F. Marrian and T. S. Stevens, *J. Chem. Soc.*, 379 (1945).
(4) We are indebted to Dr. Raymond-Hamet of Paris for supplying us with a sample of gelsemicine that he had received from Dr. T. Q. Chou.

The sparingly soluble perchlorate yielded alkaloid B, m.p. 172.6–174°, $[\alpha]^{25}D$ −158° (*c*, 1.35 in $CHCl_3$). *Anal.* Found: C, 69.77, 69.69; H, 7.52, 7.30; N, 8.57; OCH_3, 9.18; NCH_3, 4.22. Calcd. for $C_{19}H_{24}O_3N_2$: C, 69.49; H, 7.37; N, 8.53; 1 OCH_3, 9.43; 1 NCH_3, 4.57. Alkaloid B contained one C-methyl and one active hydrogen (Zerewitinow); it gave a neutral benzoyl derivative, m.p. 251–252°, $[\alpha]^{25}D$ −116° (*c*, 0.99 in $CHCl_3$). *Anal.* Found: C, 72.23; H, 6.55; N, 6.54. Calcd. for $C_{26}H_{28}O_4N_2$: C, 72.20; H, 6.53; N, 6.48. The properties of alkaloid B are quite different from those of gelsemine and of gelsemicine and the infrared absorption spectra of these three bases are quite distinct. Alkaloid B thus appears to be new and it is proposed to designate it as gelsedine. Recently Janot, Goutarel and Friedrich[5] isolated from *G. sempervirens* an alkaloid (m.p. 171°, $[\alpha]D$ −160°) which gave rise to a benzoyl derivative, m.p. 262°, $[\alpha]D$ −117°. They claimed their base to be gelsemicine and assigned to it the empirical formula $C_{19}H_{24}O_3N_2$ which is the same as that now assigned to gelsedine. The properties of gelsedine were the same as those of Janot and co-workers' gelsemicine except for the ten-degree difference in the reported melting point of the benzoyl derivatives. The ultraviolet absorption spectrum of Janot and co-workers' alkaloid resembled that of gelsemine and was the same as that of gelsedine so that the two are probably identical and both are certainly different from gelsemicine.

The basic fraction obtained from the benzoylation yielded a further base (alkaloid C) which was an oil (*Anal.* Found: C, 71.18; H, 7.00. Calcd. for $C_{21}H_{24}O_3N_2$: C, 71.57; H, 6.87), but formed a crystalline perchlorate, m.p. 250–252°. *Anal.* Found: C, 55.75; H, 5.66; N, 6.34. Calcd. for $C_{21}H_{24}O_3N_2 \cdot HClO_4$: C, 55.69; H, 5.56; N, 6.19. This base, which has an empirical formula differing from that of gelsemine by CH_2O, appears to be new.

(5) M. M. Janot, R. Goutarel and W. Friedrich, *Ann. pharm. franc.*, **9**, 305 (1951).

DIVISION OF PURE CHEMISTRY
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RECEIVED JUNE 25, 1953

A SYNTHESIS OF HYDROPEROXIDES FROM GRIGNARD REAGENTS

Sir:

The reaction of aryl and alkyl Grignard reagents with oxygen is well known and has been found to give poor yields of phenols,[1] and good yields of alcohols.[2,3] The sequence

$$RMgX + O_2 \longrightarrow ROOMgX$$
$$ROOMgX + RMgX \longrightarrow 2ROMgX$$

has been proposed[4] for this reaction and is supported by small, but significant peroxide titration values.[5]

We have found that by slow addition of alkyl Grignard reagents to oxygen-saturated ether at −75°, the intermediate ROOMgX can be ob-

(1) F. Bodroux, *Compt. rend.*, **136**, 158 (1903).
(2) L. Bouveault, *Bull. soc. chim.*, [3] **29**, 1051 (1903).
(3) M. T. Goebel and C. S. Marvel, THIS JOURNAL, **55**, 1693 (1933).
(4) C. W. Porter and C. Steele, THIS JOURNAL, **42**, 2650 (1920).
(5) H. Wuyts, *Bull. soc. chim. Belg.*, **36**, 222 (1927).