hours at average fluxes of 3.2×10^{12} and 2.6×10^{12} neutrons/cm.^{2}/sec., respectively, and the products examined for their radioactive vitamin content. The nominal specific activities due to radioactive cobalt produced were 0.18 and 1.7 μc./mg., as determined by γ-ray comparison with a Bureau of Standards Co^{60} standard. The presence of P^{32} was also demonstrated by β-ray range and decay measurements.

Absorption spectra of the capsule contents in aqueous solution indicated losses of B_{12} amounting to 5 and 19%, respectively, for the 2-days and 6-day irradiated samples. That the induced radioactivity was not necessarily associated with residual vitamin was demonstrated by treating the aqueous solutions buffered to *p*H 6 with a carbon tetrachloride solution of dithizone to remove free cobalt, and by extracting free B_{12}-like materials with benzyl alcohol after addition of $(NH_4)_2SO_4$ to the aqueous solutions. Thus extraction of aliquots of the 2-day sample with dithizone and with benzyl alcohol showed that 80% of the radioactivity was present as free cobalt and that only 20% was extractable by the alcohol. These measurements were made by gamma ray counting of evaporation residues with a thin window Geiger tube through a thick aluminum absorber (848 mg./cm.^{2}), and represent only Co^{60}. The sample irradiated for 6 days contained only $\cong$26% of the radioactivity in the form of free cobalt, which is interpreted as indicating secondary reactions of liberated cobalt, such as complex formation or isotope exchange, with decomposition products from the vitamin.

The bulk of the latter (6-day) sample was treated with cyanide to convert any B_{12a} or other analogs possibly present to vitamin B_{12}, and subjected to extensive purification involving, in succession free cobalt removal, solvent extraction, precipitation, chromatography on alumina and crystallization from acetone. At no stage was a material with constant specific activity obtained. Thus the product eluted with methanol from alumina with an over-all yield of 65% had a low activity of 0.069 μc./mg.; and the crystallized material, obtained in 49% yield, had the still lower specific activity of 0.045 μc./mg. The latter material was subjected further to an 8-tube countercurrent distribution in the system water–benzyl alcohol. Color and radioactivity measurements are shown in Table I as per cent. of total. These figures are based on the absorbancies of solutions at 3610 Å., and upon the β-ray activities of evaporation residues determined with a thin window Geiger tube after decay of P^{32} activity. It is obvious from these figures that no correlation exits between the vitamin and radioactivity concentrations. Although the color distribution was essentially normal, the bulk of the radioactivity concentrated in the first tube. The nominal specific activity of the contents of the fourth tube, in which pure vitamin concentrates, was down to 0.015 μc./mg.; and even this value is evidently fictitiously high. Obviously our purification procedures have reduced the specific activity to an insignificant figure. In all likelihood, additional treatment would lead to still further reductions in specific activity. It is clear from these results that the extent of activation of vitamin B_{12} is negligible, if it occurs at all, under the irradiation conditions employed.

TABLE I

COUNTERCURRENT DISTRIBUTION OF CRYSTALLIZED PRODUCT

Tube	Theoret. % of total	Color distribution Ratio[a]	Color distribution % of total	Radioactivity distribution Ratio[a]	Radioactivity distribution % of total
1	1.44	4.75	3.18	41.0	50.4
2	8.38	1.53	7.88	4.75	10.6
3	21.0	1.24	17.8	1.85	8.04
4	29.1	1.16	24.6	1.20	8.42
5	24.3	1.04	22.2	0.93	7.54
6	12.1	0.89	13.9	.71	5.76
7	3.37	0.58	6.7	.40	4.48
8	0.40	0.24	3.7	.09	5.34

[a] Ratio of water to benzyl alcohol concentrations.

Smith[8] has irradiated 20 mg. of vitamin B_{12} for 4 weeks at a low neutron flux of 0.5×10^{11} neutrons/cm.^{2}/sec. From an initial specific activity of 0.2 μc./mg., he reports the isolation of vitamin B_{12} fractions with a specific activity of 0.0065–0.012 μc./mg. The countercurrent test was not applied. This corresponds to a specific activity retention of 3.3–6% as compared to the low value of $<<0.9$% attained in our case after subjecting similar material to countercurrent distribution. This difference, if real, may reflect differences in neutron energies utilized. In any case, a retention[7] of 80% as reported elsewhere for the neutron irradiation of vitamin B_{12} is highly unlikely.

(8) E. Lester Smith, *Biochem. J.*, **52**, 384 (1952).

PROCESS RESEARCH AND DEVELOPMENT DIV.
MERCK & CO., INC.
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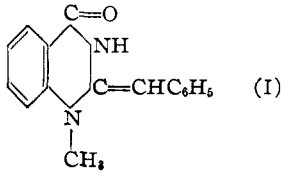
COMMUNICATIONS TO THE EDITOR

CONSTITUTION AND SYNTHESIS OF GLYCOSIN, THE NEW ALKALOID OF *GLYCOSMIS PENTAPHYLLA*, RETZ. DC.

Sir:

Glycosin, $C_{16}H_{14}N_2O$, m.p. 155°, one of the alkaloids of *Glycosmis pentaphylla*,[1-3] Retz, DC. has been proved to be a 1-methyl-4-quinazolone compound from the studies of its ultraviolet and infrared absorption spectra and hydrolysis characteristics.[1] On catalytic hydrogenation glycosin forms a dihydro derivative, $C_{16}H_{16}N_2O$, m.p. 196° (*Anal.* Calcd. for $C_{16}H_{16}N_2O$: C, 76.19; H, 6.35; N, 11.11. Found: C, 76.32; H, 6.31; N, 11.23), which also has been obtained from the base by its reduction with $LiAlH_4$ in tetrahydrofuran at room temperature. On reduction with $LiAlH_4$ in boiling tetrahydrofuran glycosin yields, however, a product different from dihydroglycosin. Further investigation of the reduction product is in progress. On ozonolysis and on oxidation with periodic acid glycosin liberates benzaldehyde which has been identified as its 2,4-dinitrophenylhydrazone, m.p. 235°. From the collective review of these experimental results it is now established that glycosin is a 2-benzylidine-1-methyl-4-quinazolone (I)



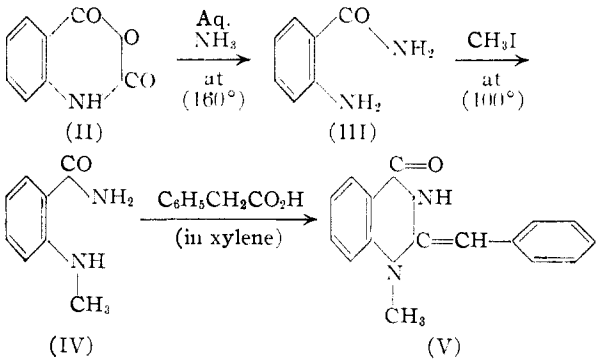
(I)

(1) Asima Chatterjee and S. Ghosh Majumdar, *Science and Culture*, **18**, 604 (1953).

(2) Asima Chatterjee and S. Ghosh Majumdar, *ibid.*, **18**, 505 (1953).

(3) Asima Chatterjee and S. Ghosh Majumdar, *ibid.*, **17**, 306 (1952).

The structure (I) for glycosin has been confirmed by synthesis from isatoic anhydride (II), m.p. 240°, which on treatment with aqueous ammonia (30%) at 160° gave anthranilide (III), m.p. 108° (yield, 90%). The latter on heating with methyl iodide at 100° for 6 hours in a sealed tube gave N-methylanthranilamide (IV), m.p. 159° (yield, 90%). N-Methylanthranilamide when refluxed with a molar proportion of phenylacetic acid in xylene (dried over sodium) with excess of phosphorus pentoxide for one hour produced glycosin, $C_{16}H_{14}N_2O$, m.p. 155° (I) (yield, 55% of the theoretical). *Anal.* Calcd. for $C_{16}H_{14}N_2O$: C, 76.80; H, 5.60; N, 11.20; NMe, 6.00; M.W., 250. Found: C, 76.45; H, 5.45; N, 11.34; NMe, 6.24; M.W., 244 by Rast and 247 by chloroplatinate method.



(II) (III) (IV) (V)

Synthetic and natural glycosin showed no depression in their mixed melting points and also in the mixed melting points of their salts. On ozonolysis and on oxidation with periodic acid synthetic glycosin produces benzaldehyde like the natural product and their infrared absorption spectra are exactly identical. Synthesis of glycosin, its ozonolysis and periodic acid oxidation experiments have enabled the authors to settle the molecular formula of glycosin as $C_{16}H_{14}N_2O$ and not $C_{15}H_{12}N_2O$, a point which cannot be decided from the analysis of the base and its salts.[1]

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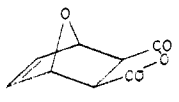
S. GHOSH MAJUMDAR

RECEIVED JULY 22, 1953

CIS-ADDITION IN THE BROMINATION OF A BICYCLIC OLEFIN

Sir:

Reaction of *exo-cis*-3,6-endoxo-Δ^4-tetrahydrophthalic anhydride (I) with bromine in oxygen-free methylene chloride yields two saturated dibromides: IIA (55%), m.p. 163°; *Anal.* Calcd. for $C_8H_6O_4Br_2$: C, 29.48; H, 1.86; Br, 49.04. Found: C, 29.56; H, 1.96; and IIB (36%), m.p. 331°; *Anal.* Found: C, 29.55; H, 1.99; Br, 48.99.



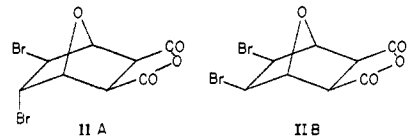
I

Each was degraded *via* the sequence anhydride → amidic acid → imide. IIA gave an imide (IIIA), m.p. 221° (d.); *Anal.* Calcd. for $C_8H_7O_3NBr_2$: C, 29.56; H, 2.17; N, 4.31. Found: C, 29.72; H, 2.07; N, 4.17. IIB gave an imide (IIIB), m.p. 297° (d.); *Anal.* Found: C, 29.85; H, 2.14; N, 4.28. Either IIIA or IIIB with zinc in acetic acid gave the known[1] *exo-cis*-3,6-endoxo-Δ^4-tetrahydrophthalimide (IV). The *endo*-isomer[1] (V) of IV is stable under the debromination conditions.

Partial optical resolution of the acid (VI) of IIA *via* the quinine salt gave (−)-VI, $[\alpha]_D$ −77.5°; *Anal.* Calcd. for $C_8H_8O_5Br_2$: C, 27.93; H, 2.34. Found: C, 28.04; H, 2.07. The infrared spectra of (−)-IIA, $[\alpha]_D$ −56°, and of (−)-dimethyl ester of VI, $[\alpha]_D$ −73°, were identical with those of the corresponding racemates.

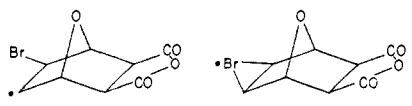
Partial optical resolution of the half-methyl ester (VII) prepared from IIB gave (+)-VII $[\alpha]_D$ +2.5°, and (−)-VII ,$[\alpha]_D$ −3.3°; *Anal.* Calcd. for $C_9H_{10}O_5Br_2$: C, 30.19; H, 2.81. Found: C, 30.41; H, 3.16, infrared spectrum identical with that of racemic VII. Methylation of (+)-VII or (−)-VII gave *optically inactive* dimethyl ester, m.p. 200°, alone or mixed with a sample prepared from IIB; *Anal.* Calcd. for $C_{10}H_{12}O_5Br_2$: C, 32.28; H, 3.25. Found: C, 31.92; H, 3.29. Hydrolysis of (+)-VII gave optically inactive acid (VIII) m.p. 331°; *Anal.* Found: C, 28.18; H, 2.39.

IIA is racemic and therefore has the *trans*-dibromide configuration. IIB is a *meso* compound and therefore has the *cis*-dibromide configuration. By application of the *exo*-addition rule,[2] the *exo*-configuration is assigned to the bromines of IIB.



IIA IIB

Formation of IIB appears to occur largely by a free radical reaction. In darkness or in polar solvents (acetic acid, ethyl acetate), bromination of I gives *ca.* 90% of IIA and 0% of IIB. The repulsive non-bonded interaction[3] of the "eclipsed" bromines of IIB should result in a higher activation energy[4] for formation of IIB than for IIA in a mechanism involving a random attack on a radical intermediate such as IX. Also, attack on a cyclic radical[5] (X) would lead largely to IIA.

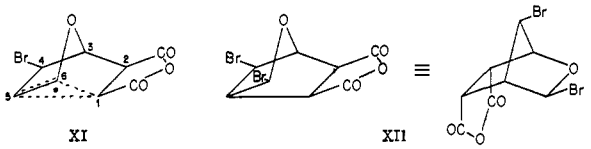


IX X

The occurrence of a high proportion of *cis*-bromination despite these considerations implies the operation of some powerful stereo-electronic demand. This may be attributable to the intermediate formation of (i) the bridged-radical (XI) re-

(1) H. Kwart and I. Burchuk, THIS JOURNAL, **74**, 3094 (1952).
(2) K. Alder and G. Stein, *Ann.*, **515**, 185 (1935).
(3) O. Bastiansen and O. Hassel, *Tids. Kjemi, Bergvesen Met.*, **6**, 96 (1946); O. Hassel and B. Ottar, *Acta Chem. Scand.*, **1**, 929 (1947).
(4) M. G. Evans and M. Polanyi, *Trans. Faraday Soc.*, **34**, 11 (1938).
(5) H. L. Goering, P. I. Abell and B. F. Aycock, THIS JOURNAL, **74**, 3588 (1952).

quiring stereospecific approach of bromine to C_6 from the *exo*-direction, and or (II) the unstable α-bromoether (XII), ionic rearrangement of which to IIB is in precise steric and electronic analogy to the change camphene hydrochloride → isobornyl chloride.[6]



XI XII

The formation of *cis*-dibromide by either or both of these paths appears to be unique in the literature.

(6) (a) H. Meerwein and K. van Emster, *Ber.*, **53**, 1815 (1920); **55**, 2500 (1922); (b) P. D. Bartlett and I. Pöckel, THIS JOURNAL, **59**, 820 (1937); **60**, 1585 (1938); (c) T. P. Nevell, E. de Salas and C. L. Wilson, *J. Chem. Soc.*, 1188 (1939).

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF SOUTHERN CALIFORNIA JEROME A. BERSON
LOS ANGELES 7, CALIFORNIA RONALD SWIDLER

RECEIVED JULY 1, 1953

PARTICIPATION OF ATP AND COENZYME A IN THE ENZYMATIC DECARBOXYLATION OF MALONIC ACID[1]

Sir:

Malonic acid was previously shown to be an intermediate metabolite of uracil degradation by bacterial enzymes.[2,3] More recently decarboxylation of malonic acid was observed with dried bacterial cells and crude extracts.[4] It has now been found that the enzymatic decarboxylation of malonic acid requires adenosinetriphosphate (ATP) and coenzyme A (CoA) and an activated form of malonate is proposed as an intermediate.

Pseudomonas fluorescens strain TR-23,[5] a strictly aerobic microörganism, was grown for about 20 hours at 26°, with constant shaking, in a medium containing 1% NH_4Cl, 0.5% disodium malonate, 0.15% K_2HPO_4, 0.05% KH_2PO_4, 0.02% $MgSO_4 \cdot 7H_2O$ and 0.1% Difco yeast extract. Cell-free extracts were prepared by grinding the washed cells with alumina (Alcoa A-301) in the presence of reduced glutathione (1.5 mg. of the sodium salt per g. of wet cells), extracting with 6 parts of 0.02 *M* phosphate buffer (*p*H 7.0), and centrifuging at 25,000 × *g* for 30 minutes.

A reaction mixture (2.0 ml.) containing 0.1 ml. of the crude extract (1.43 mg. protein), 100 μM. KF, 20 μM. reduced glutathione (sodium salt), 10 μM. $MgCl_2$, 200 μM. sodium acetate buffer (*p*H 5.8), 100 units CoA, 10 μM. ATP (sodium salt), 50 γ cocarboxylase and 100 μM. sodium malonate was incubated under pure nitrogen at 30° for 30 minutes. In the complete system 28.8 μM. of carbon dioxide was evolved. When ATP and CoA were omitted, only 1.9 μM. of carbon dioxide was produced. Pretreatment of the extracts with both Dowex-1[6] and charcoal[7] caused a more pronounced difference. With 0.1 ml. of the treated extract (0.86 mg. protein) the complete system yielded 13.5 μM. of carbon dioxide, whereas when either CoA, or ATP or both were omitted, 2.6, 0.9 and 0.2 μM. of carbon dioxide was produced, respectively. Neither Mg^{++} nor cocarboxylase affected the rate of the reaction under these conditions. There was no carbon dioxide production when malonate was omitted or the extract was treated at 100° for 5 minutes.

A reaction mixture (prepared as described above but with tris-(hydroxymethyl)-aminomethane buffer, *p*H 7.0, instead of acetate buffer) containing 1.0 ml. of the crude extract and 200 μM. of hydroxylamine, yielded 7.4 μM. of hydroxamic acid derivatives[8] in the presence of ATP and CoA, whereas only 0.15 μM. was formed in the absence of the added cofactors. These hydrosamic acid derivatives were tentatively identified by paper chromatography (Whatman No. 3 with water-saturated butanol as solvent[9]) as (1) acethydroxamic acid (R_f: 0.51–0.53) and as (2) malonmonohydroxamic acid[10] (R_f: 0.36–0.38).

Thus the mechanism of malonate decarboxylation appears to involve activation of malonate (probably as malonyl CoA) as a primary step, analogous to the mechanism of succinate decarboxylation recently proposed for anaerobic microörganisms.[11,12] It has not yet been established whether the decarboxylation occurs at the activated carboxyl group to form an active one carbon compound and free acetate or whether the other carboxyl group is decarboxylated to produce active acetate and carbon dioxide. Since crude extracts were found to form hydroxamic acid derivatives from acetate, propionate, and succinate under the conditions described above, purification of the enzymes involved appears to be necessary to elucidate this point.

DEPARTMENT OF MICROBIOLOGY
WASHINGTON UNIVERSITY SCHOOL OF MEDICINE
ST. LOUIS, MISSOURI OSAMU HAYAISHI[13]

RECEIVED JUNE 16, 1953

(1) This investigation was supported in part by a research grant (G3727) from the National Institutes of Health, Department of Health, Education and Welfare.

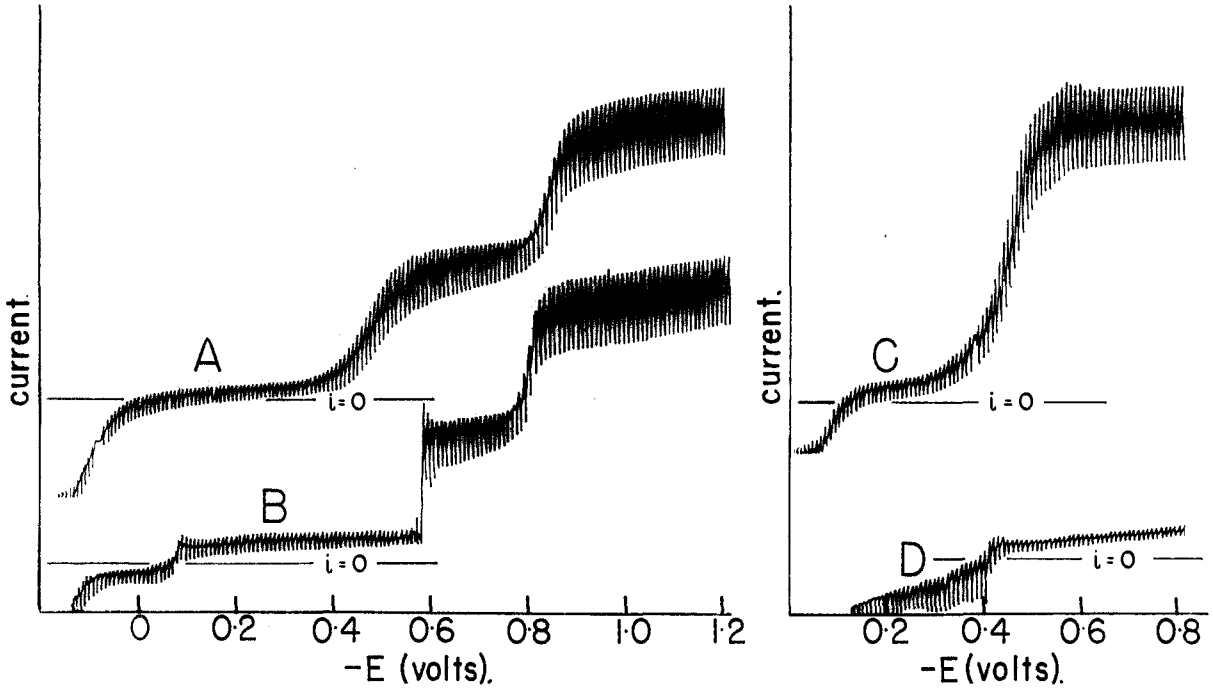
(2) O. Hayaishi and A. Kornberg, *J. Biol. Chem.*, **197**, 717 (1952)
(3) F. J. S. Lara, *J. Bact.*, **64**, 279 (1952).
(4) C. T. Gray, *ibid.*, **63**, 813 (1952).
(5) O. Hayaishi and R. Y. Stanier, *J. Biol. Chem.*, **195**, 735 (1952).
(6) H. Chantrenne and F. Lipmann, *J. Biol. Chem.*, **187**, 757 (1950).
(7) R. K. Crane and F. Lipmann, *ibid.*, **201**, 235 (1953).
(8) F. Lipmann and L. C. Tuttle, *ibid.*, **159**, 21 (1945).
(9) Incubation mixtures were treated with Dowex 50 (H^+ form) and then treated according to E. R. Stadtman and H. A. Barker, *J. Biol. Chem.*, **184**, 769 (1950).
(10) The author is indebted to Dr. David Lipkin for suggestions in preparing synthetic malonmonohydroxamic acid.
(11) E. A. Delwiche, E. F. Phares and S. F. Carson, *Federation Proc.*, **12**, 194 (1953).
(12) H. R. Whitley, THIS JOURNAL, **75**, 1518 (1953).
(13) The excellent technical assistance of Mrs. Natalie A. Fraser is gratefully acknowledged.

A MODEL FOR THE CONFIGURATION OF SULFHYDRYL GROUPS IN PROTEINS

Sir:

The differing reactivity of protein –SH groups and, especially, the marked increase in their reactivity upon denaturation of the protein, has been the subject of much speculation. We wish to report two sets of observations which suggest an explanation for this phenomenon.

In the first series of experiments three cysteine-



Curve A: PCG, 2.2 × 10^{-4} *M*; C_6H_5HgOH, 2.2 × 10^{-4} *M*; acetate buffer, *p*H 5.9, 0.05 *M*; KNO_3, 0.1 *M*. Curve B: PCV, 2.2 × 10^{-4} *M*; C_6H_5HgOH, 2.2 × 10^{-4} *M*; acetate buffer, *p*H 5.9, 0.05 *M*; KNO_3, 0.1 *M*.

Curve C: PCG, 2.4 × 10^{-4} *M*; Salyrgan, 2.4 × 10^{-4} *M*; acetate buffer, *p*H 4.0, 0.05 *M*; KCl, 0.01 *M*. Curve D: PCV, 2.4 × 10^{-4} *M*; Salyrgan, 2.4 × 10^{-4} *M*; acetate buffer, *p*H 4.0, 0.05 *M*; KCl, 0.1 *M*.

Fig. 1.—Polarograms which are identical with curves A and C are obtained when compound GSH is substituted for compound PCG.

containing peptides, *i.e.*, L-glutamyl-L-cysteinyl-glycine (GSH), phenacetyl-L-cysteinyl-glycine (P-CG)[1] and phenacetyl-L-cysteinyl-D-valine (PCV)[2] were used. These peptides can be transformed quantitatively into mercaptides of the structure RSHgR′ or RSAg by interaction with an equivalent of an organic mercury compound or silver. The phenylmercuric, Salyrgan (sodium-*o*-(γ-hydroximercuri - γ - methoxypropylcarbamyl) - phenoxyacetate) and silver mercaptides of GSH, PCG and PCV were therefore subjected to polarographic analysis. The reduction of the metal out of the mercaptide proceeded normally with all three mercaptides of GSH and PCG.[3] The waves (curves A and C) were analogous to those of the corresponding mercaptides of simpler thiols, such as cysteine, thioglycolic acid and thioethanolamine.[4] The mercaptides of PCV, on the other hand, showed a striking difference. The silver and Salyrgan mercaptides were found to be essentially irreducible (curve D). In the case of the phenylmercuric mercaptide to PCV, the initial part of the first reduction wave is seen to be missing (curve B) and a sharp rise in current occurs from the base line to the diffusion plateau, at a potential corresponding to the end of the mercaptide waves of GSH or PCG. In the presence of 4.4 *M* urea, a well-known protein denaturing agent, this "inhibited" wave became more similar to the corresponding waves of GSH or PCG (curve A).

It was therefore of interest to compare quantitatively the effect of "denaturing concentrations" of urea on the nitroprusside color of thiols of varying reactivity. It was found that, whereas sodium sulfide and ethyl mercaptan gave the same nitroprusside color in 6 *M* urea and in water, this was not the case with cysteine or the three peptides. The nitroprusside color in 6 *M* urea was about 50% higher than that in water with cysteine and in the case of the three peptides the increase amounted to over 100%. The increase in the reactivity of these thiols in the presence of urea suggests that intramolecular S–H–N bonds stabilize the –SH group through the formation of a five or six membered ring. Hydrogen bonding to sulfur has previously been postulated by Cecil[5] to account for the difference in the reactivity of the –SH group in cysteine and glutathione.

The polarographic results with PCV suggest that a further decrease in the reactivity of the –SH group can be brought about when hydrogen bonding brings a neighboring aliphatic side chain, such as the isopropyl group of valine, into close proximity of the –SH group. Thus a gradation of reactivity of protein –SH groups becomes easily conceivable,

(1) Synthesized by Drs. L. C. King and F. H. Suydam, THIS JOURNAL, **74**, 5499 (1952).

(2) Donated by Dr. K. Folkers, F. W. Holly, E. W. Peel, E. L. Lux and K. Folkers, *ibid.*, **74**, 4539 (1952).

(3) The silver mercaptide of PCG could not be polarographed, since it was insoluble under the conditions used.

(4) R. Benesch and R. E. Benesch, THIS JOURNAL, **73**, 3391 (1951); *Arch. Biochem.*, **38**, 425 (1952).

(5) R. Cecil, *Biochem. J.*, **47**, 572 (1950).

depending in the main on hydrogen bonding, with or without steric hindrance by branched aliphatic side chains. The hindered reduction of the mercaptides of PCV actually constitutes an intermediate case, as we have found that the phenylmercuric mercaptides of ovalbumin are completely irreducible polarographically.

The ideas put forward here represent a compromise between chemical and physical theories which have been proposed to account for the "masking" of protein –SH groups, since it is postulated that a chemical link (hydrogen bonding) leads to steric hindrance by branched aliphatic side chains.

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REINHOLD BENESCH

RECEIVED JUNE 11, 1953

ON THE POLAROGRAPHIC REDUCTION WAVE OF DEHYDROASCORBIC ACID

Sir:

Since 1938[1] the polarographic oxidation wave of ascorbic acid has been well known among polarographists; however, the reduction wave of dehydroascorbic acid has not yet been found. So we attempted to obtain this reduction wave by using relatively high concentrations of dehydroascorbic acid in polarographic solutions.

For that purpose crystalline dehydroascorbic acid was prepared, using Pecherer's method.[2] Our polarographic experimental conditions were as follows:

Concn. of dehydroascorbic acid, 0.025∼ 0.1 *M*
*p*H range 2∼5 in McIlvaine's buffer solution
Temperature range 10∼50° (at 5° intervals)

On the other hand, we also used dehydroascorbic acid which was not crystallized but was prepared by oxidizing ascorbic acid in buffer solutions with equimolar amounts of iodine. Consequently these solutions became more acidic, then we neutralized them partly with some portion of 1 *N* NaOH and used them as polarographic solutions without excluding I^- ions. These I^- ions showed polarographic oxidation wave but did not disturb the reduction wave of dehydroascorbic acid. The final *p*H of the solution was measured by means of a glass electrode.

The reduction wave of dehydroascorbic acid was very small at room temperature (about $^1/_{1000}$ of the expected diffusion current of dehydroascorbic acid) and had all the typical characteristics of the kinetic current which was prominently examined and explained by several authors when they had experimented with formaldehyde[3,4] or aldoses.[5,6] That is, characteristics such as the fact that the wave height of dehydroascorbic acid remains constant, independently of the height of the mercury reservoir (Fig. 1) and the temperature coefficient of this wave height is extraordinarily large (Fig. 2).

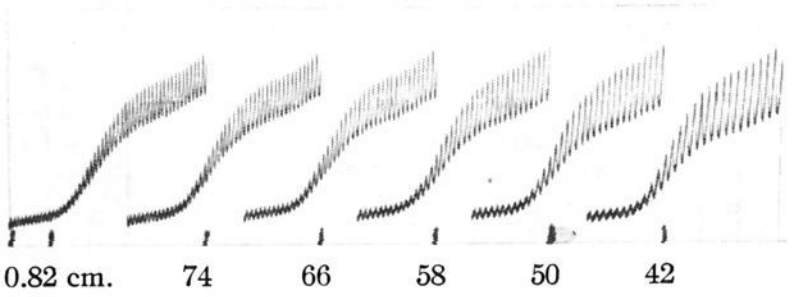

Fig. 1.—Constancy of the wave height of dehydroascorbic acid, independently of the height of the mercury reservoir: 0.1 mole of dehydroascorbic acid in McElvaine buffer solution, $S = ^1/_5$, *p*H 2.8, 25°.

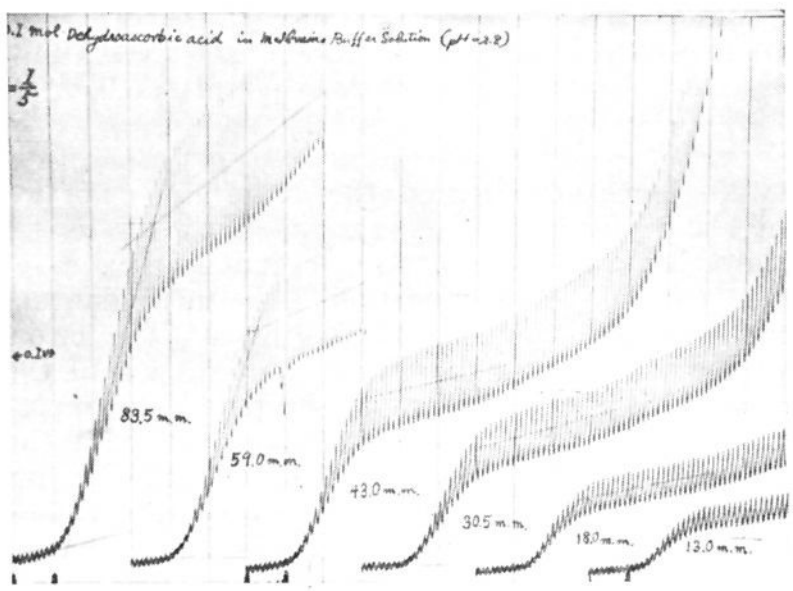

Fig. 2.—Increase of wave height of dehydroascorbic acid with increasing temperature: galv. sensitivity, 5.50×10^{-9} amp.; $m = 1.323$ mg./sec.; $t = 2.7$ sec./drop; 0.1 mole of dehydroascorbic acid in McElvaine buffer solution, *p*H 2.8; $S = ^1/_5$.

The above described results may be reasonably explained, if we assume that the electroactive form of dehydroascorbic acid (unhydrated form) is scarce in aqueous solutions and its limiting current is controlled practically by the rate of the dehydration of the hydrated dehydroascorbic acid. Also this assumption does not seem to conflict with others' reports in which they assumed that the equilibrium between hydrated and unhydrated dehydroascorbic acid would be much shifted toward the hydrated form.[7,8]

From the relationship between the temperature and the limiting current of the reduction wave we obtained the activation energy of the dehydration reaction of hydrated dehydroascorbic acid. The activation energy is about 13 kcal., which was proved to be independent of *p*H.

The fact that the reduction product of dehydroascorbic acid at the dropping mercury electrode is ascorbic acid was polarographically substantiated after the controlled potential electrolysis[9] of dehydroascorbic acid.

(1) E. Kodicek and K. Wenig, *Nature*, **142**, 38 (1938).
(2) B. Pecherer, THIS JOURNAL, **73**, 3827 (1951).
(3) K. Vesely and R. Brdicka, *Collection Czechoslov. Chem. Communs.*, **12**, 313 (1947).
(4) R. Bieber and G. Trümpler, *Helv. Chim. Acta*, **30**, 706 (1947).
(5) R. Brdicka and K. Wiesner, *Collection Czechoslov. Chem. Communs.*, **12**, 138 (1947).
(6) P. Delahay and J. E. Strassner, THIS JOURNAL, **74**, 893 (1952).
(7) Z. Vavrin, *Collection Czechoslov. Chem. Communs.*, **14**, 367 (1949).
(8) R. Brdicka and P. Zuman, *ibid.*, **15**, 766 (1950).
(9) J. J. Lingane, *Trans. Faraday Soc. Discussion*, **1**, 203 (1947).

TABLE I

HALF-WAVE POTENTIALS OF DEHYDROASCORBIC ACID IN McELVAINE BUFFER SOLUTION AT 25°

Concentration of dehydroascorbic acid, 0.025 *M*

*p*H	2.2	2.66	2.96	3.48
$\pi^1/_2$ (*vs.* N. C. E.)	−0.350	−0.372	−0.392	−0.410
*p*H	3.80	4.22	4.63	5.04
$\pi^1/_2$ (*vs.* N. C. E.)	−0.432	−0.450	−0.462	−0.480

The significance of these half wave potentials will be discussed later.

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SAKAI CITY, JAPAN — TAMOTU WASA

RECEIVED JULY 15, 1953

STUDIES ON PITUITARY ADRENOCORTICOTROPIN. VI. AN N-TERMINAL SEQUENCE OF CORTICOTROPIN-A

Sirs:

We have investigated the N-terminus of two highly purified ACTH preparations by the use of the DNFB method of Sanger and also by a recently developed modification of the thiohydantoin method of Edman.[1] As used by us, the latter procedure involves the direct identification of the hydantoin by paper chromatography.[2] In applying this technique to the characterization of the fractions arising from the chromatography of unhydrolyzed hog pituitary extracts on XE-97 resin,[3] it was found that the slow-moving active peak, designated Type ID, showed a single thiohydantoin corresponding to the amino acid, serine. (By contrast, the inactive material passing directly through the column, designated Type IA, gave several different thiohydantoins.) The stepwise degradation of Type ID material was continued by a second application of the Edman reaction and again a single thiohydantoin was detected, this time corresponding to the amino acid, tyrosine. Further application of the step-wise degradation technique gave equivocal results at the third position and therefore was discontinued.[4]

When the apparently pure unhydrolyzed ACTH, designated Corticotropin-A,[5] became available the stepwise degradation technique was again applied. Again the sequence Ser.Tyr. was obtained. In order to confirm the presence of serine at the N-terminus, Corticotropin-A was treated with dinitrofluorobenzene by the method of Sanger.[6] After acid hydrolysis of the DNP-Corticotropin-A, DNP-serine was identified in the ether extract by paper chromatography. At the same time, all of the serine was absent from the amino acid spectrum of the aqueous phase.[7]

Additional evidence for the presence in Corticotropin-A of the sequence, Ser.Tyr., has been obtained by the isolation of the dipeptide from the products of the chymotryptic digestion of Corticotropin-A. This peptide, which is a major constituent of the mixture, has an R_f value (Whatman #1) of 0.43 in the Partridge system[8] and travels at a rate intermediate between tyrosine and serine in an *s*-butyl alcohol/3% ammonia system.[9] Complete acid hydrolysis gave only serine and tyrosine and digestion for 24 hours with 5% carboxypeptidase resulted in complete hydrolysis to serine and tyrosine. In order to confirm the sequence of the amino acids in the dipeptide, it was treated with DNFB and hydrolyzed. By paper chromatography of the ether extract in two systems, one developed by us,[10] and the other the *t*-amyl alcohol solvent of Blackburn and Lowther,[11] serine was identified as the terminal residue. Chromatography of the aqueous layer in *t*-amyl alcohol showed no colored DNP-amino acids. Upon treatment of the paper with ninhydrin, the characteristic greyish-blue color of O-DNP-tyrosine was readily discernible, at an R_f corresponding to that of the reference compound run on the same sheet.

Thus it appears by a combination of chemical and enzymatic evidence that an N-terminal sequence of Corticotropin-A is Ser.Tyr. Cleavage of the peptide chain to form the dipeptide Ser.Tyr. is consistent with classical concepts of the specificity of chymotrypsin.[12]

Acknowledgment.—The authors wish to acknowledge the technical assistance of Mr. A. Gross.

(1) P. Edman, *Acta Chem. Scand.*, **4**, 277 (1950).

(2) W. A. Landmann, M. P. Drake and J. Dillaha, THIS JOURNAL, **75**, 3638 (1953).

(3) W. F. White and W. L. Fierce, THIS JOURNAL, **75**, 245 (1953).

(4) During the course of our work, a portion of the same preparation was given to Dr. Sidney W. Fox of Iowa State College for sequence studies by his technique (S. W. Fox, T. L. Hurst, and K. F. Itschner, THIS JOURNAL, **73**, 3573 (1951)). His results are in agreement with ours.

(5) W. F. White, THIS JOURNAL, **75**, 503 (1953). In this publication one residue of tyrosine was inadvertently omitted from the empirical formula in the fifth paragraph.

(6) F. Sanger, *Biochem. J.*, **53**, 355 (1953).

(7) The amino acids were separated by a paper chromatographic technique (J. F. Roland and A. Gross, to be published) and were developed with ninhydrin. Thus, in addition to serine, tyrosine and lysine were also missing from their usual positions due to reactions with DNFB. However, since the α-N DNP derivatives of tyrosine and lysine were not found these two amino acids were not located at the N-terminus.

(8) *n*-Butyl alcohol:acetic acid:water (80:20:100).

(9) This system is used in an extended run of 48–60 hours with an absorbent pad attached to the bottom of the sheet. Under these conditions phenylalanine, the fastest moving amino acid, has almost reached the end of a 22-inch strip. By comparison with phenylalanine Ser.Tyr. has a rate of about 0.4.

(10) Xylene/gl. acetic acid/*p*H 6.0 phthalate buffer (0.05 *M*) in volume ratios of 10:5:4. The paper, buffered with the same buffer, was equilibrated with the lower layer for sixteen hours before development with the upper layer. This system is capable of separating the DNP derivatives of Ser, Gly, Ala, Pro, and the bis-DNP derivative of lysine from the other amino acid derivatives. It also separates DNP-isoleucine and DNP-leucine from the others, but does not distinguish between the two.

(11) *Biochem. J.*, **48**, 126 (1951).

(12) H. Neurath and G. W. Schwert, *Chem. Rev.*, **46**, 69 (1953).

W. A. LANDMANN
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CHICAGO, ILLINOIS — W. F. WHITE

RECEIVED JUNE 22, 1953

INTERCONVERSION AND DEGRADATION OF REDUCING SUGARS BY ANION EXCHANGE RESINS

Sir:

In the paper chromatogram of a hydrolysate originating from a partly methylated cellulose, a fairly strong spot corresponding to D-fructose was discovered. Since cellulose does not contain fruc-

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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PRINCETON UNIVERSITY EUGENE PACSU
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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.

DEPARTMENT OF RADIATION BIOLOGY
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SCHOOL OF MEDICINE AND DENTISTRY LEON L. MILLER
ROCHESTER, NEW YORK

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. Cohu, *Chinese J. Physiol.*, **5**, 131 (1931).
(3) W. G. C. Forsyth, S. F. Marrian and T. S. Stevens, *J. Chem. Soc.*, 579 (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
NATIONAL RESEARCH COUNCIL H. SCHWARZ
OTTAWA, CANADA LÉO MARION

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).

tained in good yield. This not only supports the proposed reaction sequence, but also offers an attractive new synthesis of hydroperoxides. Table I shows the effect on yield of some systematic variations in conditions in the oxidation of *t*-butyl MgCl.

TABLE I

EFFECT OF VARYING CONDITIONS ON THE YIELD OF *t*-BUTYL HYDROPEROXIDE FROM *t*-BUTYLMAGNESIUM CHLORIDE

Run	Normality of reagent	Temp., °C.	Time of addition, min.[a]	Yield,[b] %
1	1.62	−65	40	34.4
2	1.62	−71	120	78.4
3	0.56	−71	40	85.7
4	1.74	−69	70	45.9
5	0.53	−74	80	91.4
6	0.53	−7	80	27.9

[a] Of 50 ml. of RMgCl solution to 50 ml. of oxygen-saturated ether. [b] By titration.

TABLE II

YIELDS OF HYDROPEROXIDES BY ADDITION OF VARIOUS GRIGNARD REAGENTS TO OXYGEN-SATURATED ETHER

Grignard reagent	Normality	Yield of hydroperoxide[a]
t-Butyl MgCl	0.56	85.7
t-Amyl MgCl	.35	91.9
2-Octyl MgCl	.50	91.4[c]
Cyclohexyl MgCl	.52	66.2
Cyclohexyl MgBr	.69	30.0
Ethyl MgCl	.48	57.0
Ethyl MgBr	.54	28.2
Benzyl MgCl	.50	30.0[b]

[a] By titration. [b] B.p. 53.5/0.09 min., $n^{25}D$ 1.5352, d^{20}_4 1.120, *Anal.* 90.0%. [c] B.p. 58–59 (0.5 min.), $n^{25}D$ 1.4269, d^{20}_4 0.868, *Anal.* 91.4%.

We have investigated the scope of the reaction using optimum conditions based on experience with *t*-butyl MgCl. Table II summarizes the yields of hydroperoxides obtained from various Grignard reagents under the same conditions used for run 3 of Table I. The reaction seems general for the synthesis of primary, secondary and tertiary hydroperoxides, with alkylmagnesium chlorides giving better yields than the corresponding bromides. All the hydroperoxides were isolated and characterized with the exception of ethyl hydroperoxide, the explosive character of which is well known.

Large scale runs with several of the Grignard reagents gave similar results, and yields of isolated hydroperoxide approach the quantities indicated by titration. Treatment of *t*-butyl OOMgCl with acid chlorides and alkyl halides gave peresters and peroxides, respectively. The reaction of benzyl MgCl is especially interesting since attempts to prepare benzyl hydroperoxide by autoxidation of toluene have proved unsuccessful. This hydroperoxide is fairly stable on isolation, but is converted to benzaldehyde by short treatment with alkali.

Further work continues on aromatic and acetylenic Grignard reagents, but we are reporting these preliminary results in the hope that this synthetic method will be useful to other workers in this active field.

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CHEVES WALLING
SHELDON A. BUCKLER

RECEIVED JULY 30, 1953

THE STRUCTURE OF METOPON

Sir:

Ever since the realization that metopon (methyldihydromorphinone) has properties which make it more valuable in some respects than morphine,[1] considerable interest has attached to the elucidation of its structure, a problem related to that of the structures of the two methyldihydrothebainone isomers which are formed in the reaction of enolic derivatives of dihydrocodeinone with methylmagnesium halides.[2] One of the isomers, the sole product when the enol acetate is used, leads eventually to metopon.

It was recognized by Small[2] that the methyl group which has entered the molecule must be either in position 5 or 7 of dihydrothebainone, but repeated efforts to settle this point by studying the properties of the substances formed,[2] by degradative and synthetic studies[3] and by work on model compounds[4] have failed to give the desired answer.

We have now shown that isomethyldihydrocodeinone which is formed, by reclosure of the oxide bridge, from isomethyl dihydrothebainone is 7-methyldihydrocodeinone: Formylation of dihydrocodeinone with ethyl formate and sodium ethoxide in benzene solution gave 7-hydroxymethylene dihydrocodeinone as an amorphous amphoteric solid, m.p. 179°, $[\alpha]^{25}D$ −256.5° (water), characterized as its yellow aniline derivative, m.p. 249°, dec. Calcd. for $C_{25}H_{26}N_2O_3$: C, 74.60; H, 6.51; N, 6.96. Found: C, 74.69; H, 6.75; N, 7.14. Several attempts at direct reduction of the free hydroxymethylene compound or its esters to a methyldihydrocodeinone were unsuccessful. Reduction in acetic acid with 5% palladium on charcoal gave a phenolic substance shown to be 7-hydroxymethyldihydrothebainone, m.p. 206–206.5°, $[\alpha]^{25}D$ −39° (ethanol). Calcd. for $C_{19}H_{25}NO_4$: C, 68.86; H, 7.60; N, 4.23. Found: C, 69.00; H, 7.73; N, 4.69. The desired reduction of the hydroxymethylene group was finally effected in the following manner: Transformation into the ethylenedithioacetal by treatment with ethanedithiol and anhydrous hydrogen chloride gave the anticipated compound as an amorphous solid, m.p. 75–78°, which could be purified by chromatography. Calcd. for $C_{21}H_{25}O_3NS_2$: C, 62.51; H, 6.24. Found: C, 62.02; H, 6.63. The ethylenedithioacetal was desulfurized by refluxing in acetone with Raney nickel and the product was isolated by chromatography, yielding needles, m.p. 164°, *undepressed on admixture with an authentic specimen of isomethyldihydrothebainone*, kindly supplied by Dr. L. F. Small. The infrared spectra of the two substances were also identical. Isomethyldihydrocodeinone, agreeing in melting point and ro-

(1) See for instance L. E. Lee, *J. Pharm. Exp. Ther.*, **75**, 161 (1942); T. A. Henry, "The Plant Alkaloids," J. & A. Churchill, Ltd., London, 1949, p. 262.

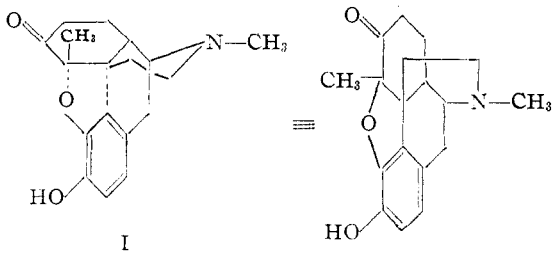
(2) L. F. Small, H. M. Fitch and W. E. Smith, THIS JOURNAL, **58**, 1457 (1936); L. F. Small, S. G. Turnbull and H. M. Fitch, *J. Org. Chem.*, **3**, 204 (1938); L. F. Small and H. M. Fitch, U. S. Patent 1,178,010 (Oct. 31, 1939).

(3) L. J. Sargent and L. F. Small, *Science*, **112**, 473 (1950); L. J. Sargent and L. F. Small, Abstracts of Papers, 118th Meeting A.C.S., Sept., 1950, p. 53N.

(4) R. I. Meltzer and J. A. King, THIS JOURNAL, **75**, 1355 (1953).

tation with the literature values, was also isolated after the desulfurization.

With the demonstration that isomethyldihydrothebainone and isomethyldihydrocodeinone have the additional methyl group at position 7, methyldihydrocodeinone and metopon are proved to be 5-methyl compounds since the isomerism of the two methyldihydrocodeinones cannot be the result of epimerism at C_7 (the two isomers are stable to base and give rise to two different enol acetates[2] and metopon can be represented by structure I.



I

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BOOK REVIEWS

The Bacteriostatic Activity of 3500 Organic Compounds for *Mycobacterium Tuberculosis Var. Hominis*. Chemical-Biological Coördination Center Review No. 4. By GUY P. YOUMANS, LEONARD DOUB AND ANNE S. YOUMANS, Parke, Davis and Company, Detroit, Michigan. Publications Office, National Research Council, 2101 Constitution Avenue N.W., Washington 25, D.C. 1953. v + 713 pp. 17.5 × 25 cm. Price, $5.00.

Despite the spectacular developments of the last ten years, the search for a specific chemotherapeutic agent for the treatment of tuberculosis continues. This is indicated by the list of over 3500 widely diversified organic compounds whose tuberculostatic properties have been evaluated. The assembled data speak much for the efforts of the authors who have evaluated these compounds, as well as for the originality and ingenuity of the chemists responsible for their preparation. This study underscores the importance to both chemists and biologists of a centralized coördination center which can assemble and codify data from many sources dealing with the preparation, evaluation and availability of potential chemotherapeutic agents applicable not only for the treatment and control of diseases in man but in animals and plants as well. In the absence of more precise knowledge of the metabolism of the tubercle bacillus, search for a specific chemotherapeutic agent is of necessity random in nature. The authors of this monograph clearly present the rationale of this approach to the subject and the methods employed. Their use of the virulent human type strain of tubercle bacillus, H37, a strain which has been widely studied by other investigators, increases the value of their study. The limitations of the *in vitro* and *in vivo* methods of determining the tuberculostatic value of various compounds are presented.

Some investigators, including the reviewer, are not in accord with the authors as to the value of mice in determining the chemotherapeutic action of chemicals or antibiotics. The administration of the compound in the feed makes it difficult to quantitate the results. The relation of chemical structure of some of the compounds to their tuberculostatic activity is discussed. Of the 3500 preparations that were investigated 184 were selected for *in vivo* tests on mice. Of these, 8 chemical compounds and 3 antibiotics showed some suppressive effects on the tuberculous process in mice. The method of classifying the 3500 compounds is described in detail. Despite the negative findings this monograph should serve as an excellent reference to both chemists and bacteriologists and should obviate the necessity of preparing and retesting many preparations included in this monograph. A point of minor criticism is the lack of description of Berkfeld and of Swinney filters. Although these names indicate a definite type of filter to the initiated, a brief description of the specification and uses of these filters would be helpful to the uninitiated. The formula and alphabetical indices are quite complete. The type is clear and the format is excellent.

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Maximum Permissible Amounts of Radioisotopes in the Human Body and Maximum Permissible Concentrations in Air and Water. National Bureau of Standards Handbook 52. Superintendent of Documents, Washington 25, D. C. 1953. iv + 45 pp. 13 × 19.5 cm. Price 20 cents.

The National Committee on Radiation Protection sponsored by the National Bureau of Standards is made up of representatives from organizations which are concerned with the safe use of ionizing radiation and radioactive materials. These organizations include several medical societies, the U. S. Armed Forces, interested Government agencies and the National Electrical Manufacturers Association. It is the responsibility of this National Committee to make health and safety recommendations.

This particular Handbook prepared by the Subcommittee on Permissible Internal Dose under the chairmanship of Karl Z. Morgan, presents recommendations for maximal permissible levels of human exposure to those radioisotopes of greatest current interest which may gain entrance to the body by absorption, inhalation or ingestion.

The levels recommended are based largely on the principal of avoiding greater radiation than the equivalent of 0.3 roentgen per week to any organ other than the skin. Because such radiation produces no easily detectible biological effects this principal is commonly regarded as conservative, particularly for periods of exposure which do not extend over many years. However, there are still so many uncertainties with respect to the absorption, retention and distribution of inhaled or ingested radioactive materials in man, as well as with respect to the equivalent effectiveness of the particulate radiations in producing chronic injury that current estimates of permissible exposure levels to many radioisotopes cannot be made with that accuracy which would finally be desirable. Nevertheless, these recommendations, carefully considered by a competent group of experts, provide a guide for health protection which is not likely to err in the direction of permitting dangerous overexposure, particularly if the suggested factor of safety is adopted. The associated problem of whether the levels recommended can be economically achieved in industry is not discussed.