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 $\simeq$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]	% of total	Radioactivity distribution Ratio[a]	% 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.

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

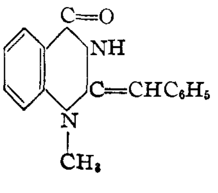
PROCESS RESEARCH AND DEVELOPMENT DIV.
MERCK & CO., INC.
RAHWAY, N. J.

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)

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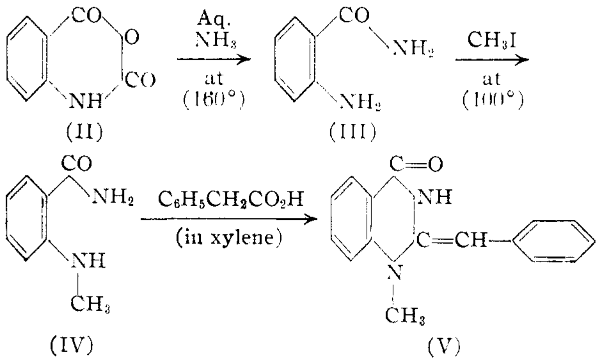
[1] Asima Chatterjee and S. Ghosh Majumdar, *Science and Culture*, **18**, 604 (1953).

[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.



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]

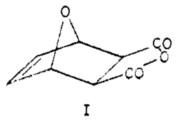
DEPARTMENT OF PURE CHEMISTRY
UNIVERSITY COLLEGE OF SCIENCE ASIMA CHATTERJEE
CALCUTTA, INDIA S. GHOSH MAJUMDAR

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.



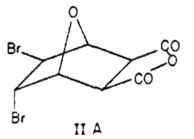
I

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.

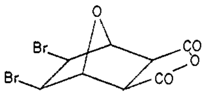
Partial optical resolution of the acid (VI) of IIA *via* the quinine salt gave (−)-VI, $[\alpha]_D$ −77.5°; *Anal.* Calcd. for $C_8H_8O_5Br_2$: C, 27.93; H, 2.34. Found: C, 28.04; H, 2.07. The infrared spectra of (−)-IIA, $[\alpha]_D$ −56°, and of (−)-dimethyl ester of VI, $[\alpha]_D$ −73°, were identical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

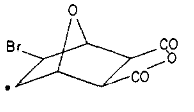


II A

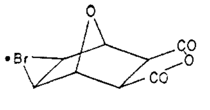


IIB

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-

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(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).