fraction II. Hydrolysis and subsequent sugar analysis by paper chromatography now showed rhamnose to be the only sugar present. The processing of the back part of the zone will be discussed in the next section. The yellow solid obtained from the combined eluates from the first (forward) part of the zone was recrystallized three times, then dried at 100° for 1 hr. It had a m.p. 183–184°, as did authentic quercitrin. No lowering of the melting point occurred on admixture of the two, and the isolated quercitrin showed no separation from authentic quercitrin by paper chromatography. One of the components (the forward portion) of fraction III from the column has, therefore, been identified as quercitrin; yield approximately 12 mg.

Properties of a Quercetin Glucoside.—The back part of the zone from the paper strips mentioned above was eluted, placed back again on fresh paper strips as before, and the strips chromatographed in 5% acetic acid. After 14 hours the new zone was about 5 cm. wide. The forward 2 cm. was cut off and discarded. The back 3 cm. was eluted and the ethyl alcohol evaporated and the solid hydrolyzed by refluxing for 2 hr. with 2% sulfuric acid. A sugar analysis made as described above showed glucose to be present as the predominant sugar with only a trace of rhamnose left from the quercitrin. The solid formed in the hydrolysis was identified as quercetin in the same manner as described for fraction I. Lack of sufficient material prevented further meaningful characterization of this compound. It may be identical with the quercetin glucoside reported, but not positively identified, from apricots.[3] It does show separation from both isoquercitrin and quercimeritrin on paper chromatograms processed in 15% acetic acid. Final yield of slightly impure product was about 5 mg.

Identification of Isoquercitrin.—The solid from flavonoid fraction IV was dissolved in 10 ml. of ethyl alcohol and put on strips by the procedure already described. The strips were processed in 15% acetic acid, air-dried for 3 hr., and the zone at R_f 0.45 was cut out and eluted with ethyl alcohol in an air-tight chamber. A total of 40 strips was processed, and after evaporation of the alcohol the solid was recrystallized eight times from hot water by cooling. The solid was dried at 110° for 1 hr. yielding a m.p. of 225–227°. It was identified as isoquercitrin by the methods previously described for this compound in *Vaccinium myrtillus*[4]; yield approximately 20 mg.

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The Preparation of Nicotinyl Chloride

BY H. N. WINGFIELD, JR., W. R. HARLAN AND H. R. HANMER

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Procedures for the preparation of nicotinyl chloride now in use seem to be modifications of the method of Meyer and Graf.[1] Salts of nicotinic acid are treated in various solvents and under varying conditions with thionyl chloride, and the product is vacuum distilled from pyridine or quinoline. The processes are tedious and often unsatisfactory.

We have now developed a more convenient method of preparation based upon the work of Adams and Ulich.[2] Potassium nicotinate suspended in benzene is treated with oxalyl chloride. The by-products, potassium chloride, carbon dioxide and carbon monoxide, are insoluble in benzene or are gases. No hydrogen chloride is formed and hence no hydrochloride. The nicotinyl chloride may be used immediately in the reaction flask in benzene solution, or siphoned off under anhydrous conditions, or distilled under vacuum after removal of the solvent.

Experimental

Sixteen and one-tenth grams of potassium nicotinate, which had been ground to pass a 100-mesh sieve and dried in an oven at 135°, and 75 ml. of anhydrous benzene was placed in a three-necked flask which was equipped with a stirrer and reflux condenser closed with a drying tube. The flask and contents were chilled in an ice-bath, and then 12.5 g. of oxalyl chloride in 25 ml. of anhydrous benzene was added at such a rate that the temperature remained low. Stirring was continued and 15 to 20 minutes after all the oxalyl chloride had been added, the unmelted ice in the bath was removed. The bath was allowed to come to room temperature, and heating was begun at a rate such that the bath began to simmer in about 30 minutes. After an additional 30 minutes heating at this temperature, the flask was allowed to cool. The cooled solution was siphoned off under anhydrous conditions and fractionated. The oily residue distilled at 75–90° (10–12 mm.). The yield was 85% or better.

Anal. Calcd. for C_6H_4ClNO: Cl, 24.70. Found: Cl, 24.82. On exposure to the air the oily chloride soon solidified to nicotinic acid hydrochloride.

Anal. Calcd. for $C_6H_5ClNO_2$: Cl, 22.01. Found: Cl, 22.09. A sample of the oily acyl chloride treated with absolute ethanol formed ethyl nicotinate, b.p. 222–224°.

The preparation was successful using 0.5 molar proportions. Sodium nicotinate did not give as good results as the potassium salt.

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The Neutron Irradiation of Crystalline Vitamin B_{12}

BY D. T. WOODBURY AND CHARLES ROSENBLUM

RECEIVED MAY 4, 1953

Vitamin B_{12} is a hexacovalent coördination complex of cobalt with the empirical formula[1,2] $C_{61-64}H_{86-92}N_{14}O_{14}PCo$. It is labeled[3,4] with radioactive cobalt by adding cobalt 60 to the fermentation medium in which it is produced. Direct activation of cobalt in crystalline vitamin B_{12} by neutron irradiation in a pile was thought to be improbable because of the relatively high temperatures of such a nuclear reactor, and because the high γ-ray recoil energy[5] involved in the formation of a cobalt 60 atom is far in excess of normal bond energies and should result in ejection of the radioactive cobalt atom. Radioactivation by exchange with the free Co^{60} atom is also unlikely because of the exchange stability[6] of the central cobalt atom.

In view of a report[7] that such direct activation was possible, two 21-mg. samples of crystalline vitamin were irradiated in evacuated, sealed quartz ampules in the Brookhaven National Laboratories pile for $\simeq$ 48 hours and 138

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(2) Roger Adams and L. H. Ulich, THIS JOURNAL, **42**, 599 (1920).
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(2) J. F. Alicino, *ibid.*, **73**, 405 (1951).
(3) L. Chaiet, C. Rosenblum and D. T. Woodbury, *Science*, **111**, 601 (1950).
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(5) A. C. Wahl and N. A. Bonner, "Radioactivity Applied to Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1951, p. 245.
(6) R. N. Boos, C. Rosenblum and D. T. Woodbury, THIS JOURNAL, **73**, 5446 (1951).
(7) R. C. Anderson and Y. Delabarre, *ibid.*, **73**, 4051 (1951).

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]	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.

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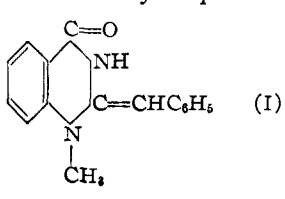
COMMUNICATIONS TO THE EDITOR

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)

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