TABLE II

N-SUBSTITUTED-N¹-NITROGUANIDINES PREPARED BY METHOD B

Amine[a]	Reaction medium aqueous ethanol % Ethanol	Vol., cc.	Reaction time, hr.	N-R-N¹-Nitroguanidine R	Yield, %
Aniline[b] *R*	50	35	0.4	Phenyl	85.0
Aniline[c] *R*	50	35	0.6	Phenyl	78.4
Aniline *R*	95	35	2.5	Phenyl	65.4
Aniline *R*	95	35	6.5	Phenyl	62.2
Aniline	75	35	1.05	Phenyl	73.5
Aniline	50	35	1.05	Phenyl	83.4
Aniline	50	35	2.0	Phenyl	83.4
o-Anisidine	51	36	3.0	*o*-Methoxyphenyl	74.4
p-Toluidine	55	50	1.5	*p*-Methylphenyl	88.8
m-Toluidine	50	35	1.5	*m*-Methylphenyl	80.0
p-Aminoacetanilide	50	35	3.5	*p*-Acetamidophenyl	88.1
2-Amino-*p*-cymene	68	55	72.0	2-Methyl-5-isopropylphenyl	26.8
2-Amino-1,4-dimethylbenzene	54	38	42.5	2,5-Dimethylphenyl	74.9
p-Chloroaniline	65	50	12.0	*p*-Chlorophenyl	76.0
m-Chloroaniline	56	40	12.0	*m*-Chlorphenyl	76.8
o-Chloroaniline *R*	52	37	4.5	*o*-Chlorophenyl	31.5
o-Chloroaniline *R*	85	38	21.5	*o*-Chlorophenyl	11.6
o-Chloroaniline *R*	85	38	48.0	*o*-Chlorophenyl	20.6

[a] Ratio of amine to methylnitrosonitroguanidine 2.65:1. [b] Ratio of amine to methylnitrosonitroguanidine 1.76:1. [c] Ratio of amine to methylnitrosonitroguanidine 1.17:1. [R] Reactions carried out at reflux temperature. All other runs recorded were carried out at a temperature of 22°.

N-Substituted-N¹-nitroguanidines from N-*n*-Butyl-N-nitroso-N¹-nitroguanidine.—Phenyl-, *p*-acetamidophenyl- and *p*-ethoxyphenylnitroguanidines were obtained in 83.4, 98.2 and 89.4% yield, respectively. Butylnitrosonitroguanidine (0.034 mole) was added in one portion to 0.09 mole of the appropriate amine dissolved in 50% aqueous ethanol. The reaction was allowed to proceed to completion at 22° when the product was recovered and identified by mixed melting point determinations.

Denitrosation of N-Methyl-N-nitroso-N¹-nitroguanidine.—To a solution of 10.89 g. (0.09 mole) of N-ethylaniline in 50 cc. of 75% ethanol was added 5 g. (0.034 mole) of methylnitrosonitroguanidine. The reaction mixture was heated for one and a half hours at 83°. Then it was cooled to *ca.* 7° and the crystals removed by filtration. After washing with ether, the crystals melted at 157°; yield 3.4 g. (84.7%). One crystallization from methanol raised the melting point to 159–161°. Admixture with an authentic sample of N-methyl-N¹-nitroguanidine did not depress the melting point.

A similar experiment using N-methylaniline in place of N-ethylaniline gave a 72% yield of methylnitroguanidine (m. p. 159–161°).

Summary

A series of N-substituted-N¹-nitroguanidines have been prepared by the reaction of primary amines with N-alkyl-N-nitroso-N¹-nitroguanidines. Denitrosation of N-methyl-N-nitroso-N¹-nitroguanidine is effected by heating with an aqueous alcoholic solution of N-methyl or N-ethylaniline.

KINGSTON, ONTARIO RECEIVED JULY 13, 1948

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, QUEEN'S UNIVERSITY]

The Nitration Products of Some Substituted 2-Nitramino-1,3-diazacylcoalkenes-2

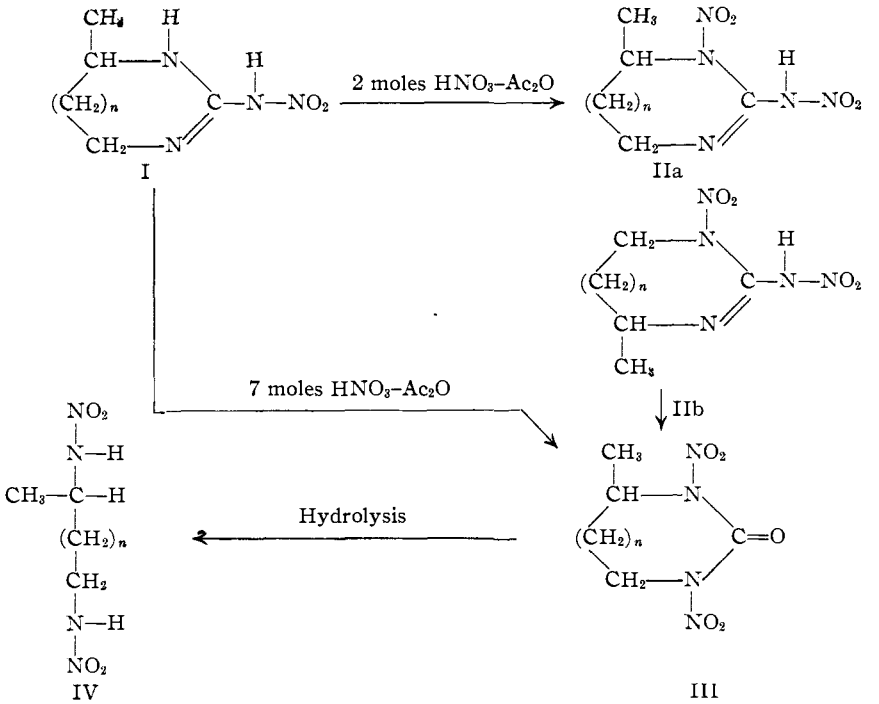
BY A. F. McKAY AND D. F. MANCHESTER

In a previous paper on the nitration products of 2-nitramino-1,3-diazacycloalkenes-2[1] it was stated that the five-membered ring compound 2-nitramino-1,3-diazacylcopentene-2 was the only one that gave a 1-nitro derivative. However 2-nitramino-1,3-diazacyclohexene-2 and 2-nitramino-1,3-diazacycloheptene-2 could be easily converted to the corresponding 1,3-dinitro-1,3-diazacycloalkanone-2. This investigation has been extended to the nitration of formerly described[2] 2-nitramino-4(or 5)-methyl-1,3-diazacyclopentene-2 I (n = 0), 2-nitramino-4(or 6)-methyl-1,3-diazacyclohexene-2 I (n = 1) and 5-hydroxy-2-nitramino-1,3-diazacyclohexene-2 V to ascertain the effect of substituents on the course of nitration.

The 2-nitramino-4(or 5)-methyl-1,3-diazacyclopentene-2 I (n = 0) on treatment with 2 mole equivalents of nitric acid in acetic anhydride gave 1-nitro-2-nitramino-4(or 5)-methyl-1,3-diazacyclopentene-2 IIa or IIb (n = 0). On the other hand the two substituted 2-nitramino-1,3-diazacyclohexenes-2 I (n = 1) and V failed to give a 1-nitro derivative when treated with nitric acid–acetic anhydride mixture or mixed acid. This substantiates the previous observations[1] on the unsubsti-

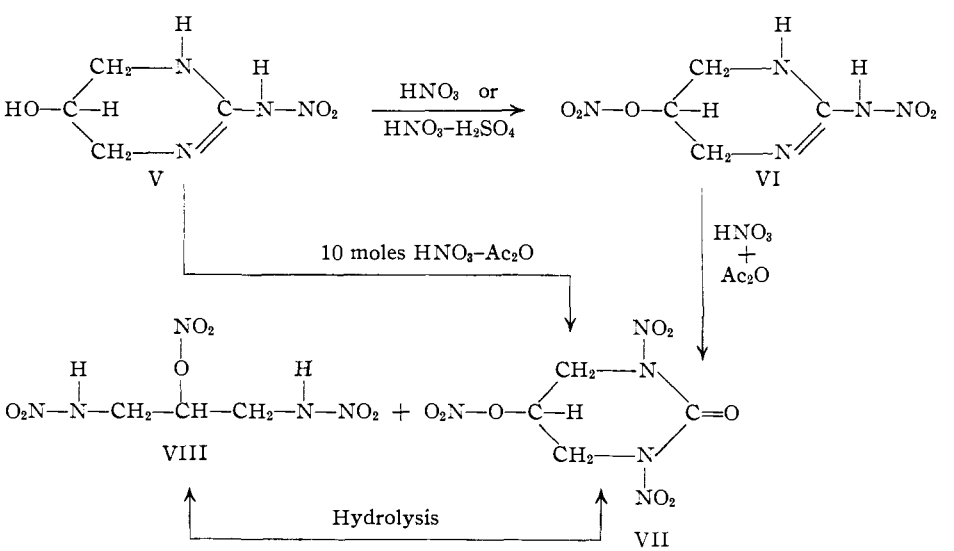
(1) A. F. McKay and G. F. Wright, THIS JOURNAL, **70**, 3990 (1948).

(2) A. F. McKay and G. F. Wright, *ibid.*, **70**, 430 (1948).



tuted 2-nitramino-1,3-diazacycloalkenes-2. When 2-nitramino-4(or 5)-methyl-1,3-diazacyclopentene-2 I (n = 0) and 2-nitramino-4(or 6)-methyl-1,3-diazacyclohexene-2 I (n = 1) were treated with seven mole equivalents of nitric acid in acetic anhydride, 1,3-dinitro-4-methyl-1,3-diazacyclopentanone-2 III (n = 0) and 1,3-dinitro-4-methyl-1,3-diazacyclohexanone-2 III (n = 1) were formed, respectively.

It was observed that the hydroxy group in 2-nitramino-5-hydroxy-1,3-diazacyclohexene-2 V could be nitrated without affecting the rest of the molecule. 2-Nitramino-5-nitroxy-1,3-diazacyclohexene-2 VI was prepared from the hydroxy compound by nitrating with nitric acid (sp. gr. 1.50) in the cold or with mixed acid. When an excess of nitric acid (ten mole equivalents) in the presence of acetic anhydride was used as the nitrating medium then a mixture of 1,3-dinitro-5-nitroxy-1,3-diazacyclohexanone-2 VII and 1,3-dinitramino-2-nitroxypropane VIII was obtained. This occurrence of ring opening in a nitrating medium has not been observed with the other compounds reported and it may have happened during the dilution of the reaction mixture with water.



The linear dinitramines were prepared from the corresponding 1,3-dinitro-1,3-diazacycloalkanones-2 by refluxing with water[3] or by dissolving in 10% aqueous sodium hydroxide solution and acidification of this solution. The following dinitramines, 1,2-dinitraminopropane IV (n = 0), 1,3-dinitraminobutane IV (n = 1), 1,3-dinitraminopropane,[4] 1,4-dinitraminobutane,[5] and 1,3-dinitramino-2-nitroxypropane VIII were prepared in 81–97% yield by one of these methods.

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Experimental[5a]

1-Nitro-2-nitramino-4(or 5)-methyl-1,3-diazacyclopentene-2.—The nitration medium was prepared by adding

(3) A. P. N. Franchimont and E. A. Klobbie, *Rev. trav. chim.*, **7**, 17, 244 (1888).

(4) A. P. N. Franchimont and E. A. Klobbie, *ibid.*, **7**, 349 (1888).

(5) M. P. J. Dekkers, *ibid.*, **9**, 97 (1890).

(5a) All melting points are uncorrected.

0.6 cc. (0.014 mole) of nitric acid (sp. gr. 1.50) with stirring to 1.5 g. (0.014 mole) of acetic anhydride at −5°. One gram (0.007 mole) of 2-nitramino-4(or 5)-methyl-1,3-diazacyclopentene-2 was added to this solution and the temperature maintained at *ca.* −5°. After a period of one hour, the solid had dissolved to give a viscous solution. On stirring this solution, crystallization occurred. These crystals were filtered off and washed with 5 cc. of ethanol. The dried product melted at 106–119° and weighed 1.2 g. (91.5%). Three recrystallizations from 95% ethanol (7 cc. per g.) gave 0.4 g. of material melting at 121.6–123°. 1-Nitro-2-nitramino-4(or 5)-methyl-1,3-diazacyclopentene-2 is very soluble in acetone and nitromethane, soluble in ethanol but quite insoluble in ether.

Anal. Calcd. for $C_4H_7N_5O_4$: C, 25.4; H, 3.70; N, 37.0. Found: C, 25.4; H, 3.61; N, 37.3.

1,3-Dinitro-4-methyl-1,3-diazacyclopentanone-2.—A solution of 3.0 cc. (0.071 mole) of nitric acid (sp. gr. 1.5) and 5.2 g. (0.051 mole) of acetic anhydride was prepared at −5°. The temperature of this solution was raised to 24° and 1 g. (0.007 mole) of 2-nitramino-4(or 5)-methyl-1,3-diazacyclopentene-2 was added portionwise over a period of ten minutes. After five minutes an exothermic reaction occurred, the solid dissolved and a colorless gas was evolved. The temperature was maintained at 24° for a period of two hours after which the reaction mixture was drowned in 50 cc. of water at 30°. This aqueous solution was cooled to 0° and the fine crystals that separated were filtered off, washed with cold water and air-dried. The crude product melted at 98–98.5°, yield 1.0 g. (75.5%). Two recrystallizations from ether (250 cc. per g.) raised the melting point to 98.5–98.9°. This compound is very soluble in acetone and nitromethane and fairly soluble in ethanol and ether.

Anal. Calcd. for $C_4H_6N_4O_5$: C, 25.3; H, 3.15; N, 29.4. Found: C, 25.3; H, 3.02; N, 28.9.

1,2-Dinitraminopropane.—One gram (0.0052 mole) of 1,3-dinitro-4-methyl-1,3-diazacyclopentanone-2 was added to 20 cc. of water and refluxed for one hour. During the first twenty minutes carbon dioxide was produced. At the end of the reaction period, the solution was concentrated to 10 cc. and cooled to 0°. The granular crystals were filtered off and washed with water. The dried product melted at 109.6–110.4° and weighed 0.7 g. (82.0%). After one crystallization from water (16 cc.), 0.5 g. of material was obtained melting at 109.8–110.4°.

Anal. Calcd. for $C_3H_8N_4O_4$: C, 21.9; H, 4.87; N, 34.1. Found: C, 22.1; H, 4.72; N, 34.6.

1,3-Dinitro-4-methyl-1,3-diazacyclohexanone-2.—The nitrating medium consisted of a solution of 2.7 cc. (0.064 mole) of nitric acid (sp. gr. 1.50) in 4.7 g. (0.046 mole) of acetic anhydride prepared at 0°. This solution was warmed to 25° and 1.0 g. (0.0063 mole) of 2-nitramino-4-(or 6)-methyl-1,3-diazacyclohexene-2 was added with stirring over a period of ten minutes. After five minutes an exothermic reaction occurred with the evolution of gas while the solid dissolved. The reaction mixture was aged for a further period of five hours at 26° after which it was cooled to 0° and poured onto 15 g. of ice. The yellow emulsion that formed initially became crystalline after a period of three hours. It was then filtered and the crystals were washed with water and air-dried. The crude product melted at 70–75.6° and weighed 1.2 g. (93%). Two crystallizations from ethyl ether gave a product melting at 84–84.7°.

Anal. Calcd. for $C_5H_8N_4O_5$: C, 29.4; H, 3.92; N, 27.4. Found: C, 29.7; H, 3.86; N, 27.0.

1,3-Dinitraminobutane.—One gram (0.0049 mole) of 1,3-dinitro-4-methyl-1,3-diazacyclohexanone-2 was dissolved in 11.3 cc. of 10% aqueous sodium hydroxide. On acidification with concentrated hydrochloric acid solution carbon dioxide was evolved. The resultant solution on cooling to −5° deposited crystals. These crystals were filtered off and washed with 2 cc. of cold water. The crude product (m.p. 81.1–83°) weighed 0.85 g. (96.9%). Two crystallizations from benzene gave crystals melting at 81.6–82.2°. A mixed melting point determination with 1,3-dinitro-4-methyl-1,3-diazacyclohexanone-2 (m. p. 84–84.7°) was depressed to 67°.

Anal. Calcd. for $C_4H_{10}N_4O_4$: C, 26.9; H, 5.61; N, 31.4. Found: C, 27.1; H, 5.42; N, 31.0.

5-Nitroxy-2-nitramino-1,3-diazacyclohexene-2. Method A.—A solution of 1 g. (0.0063 mole) of 2-nitramino-5-hydroxy-1,3-diazacyclohexene-2 in 1.9 cc. (0.045 mole) of nitric acid (sp. gr. 1.5) was prepared at 0°. This solution was added dropwise with stirring over a period of forty-five minutes to 1.75 cc. (0.033 mole) of concentrated sulfuric acid also at 0°. After aging for a further period of thirty minutes at this temperature, the reaction mixture was poured onto 25 g. of ice. A dense white precipitate formed immediately. It was removed by filtration, washed with water, and air-dried. The crude product (m. p. 158–161.8°) weighed 1.2 g. (92.8%). Two crystallizations from nitromethane (25 cc. per g.) gave 0.5 g. of product melting at 220–220.2° with decomposition.

Anal. Calcd. for $C_4H_7N_5O_5$: C, 23.4; H, 3.41; N, 34.1. Found: C, 23.6; H, 3.47; N, 33.8.

Method B.—One gram (0.0063 mole) of 2-nitramino-5-hydroxy-1,3-diazacyclohexene-2 was dissolved in 1.9 cc. (0.045 mole) of nitric acid (sp. gr. 1.50) at 0°. The solution was aged for thirty minutes at this temperature and then poured onto 15 g. of ice. The dense white precipitate was filtered off, washed with water and dried. The crude product melted at 208–210° with decomposition; yield 0.9 g. (70%). One crystallization from water (100 cc. per g.) raised the melting point to 221–221.6° with decomposition. This melting point was not depressed on admixture with an authentic sample of 2-nitramino-5-nitroxy-1,3-diazacyclohexene-2.

1,3-Dinitro-5-nitroxy-1,3-diazacyclohexanone-2 and 1,-3-Dinitramino-2-nitroxypropane.—A solution of 2.8 cc. (0.066 mole) of nitric acid (sp. gr. 1.50) in 4.7 g. (0.046 mole) of acetic anhydride was prepared by adding the nitric acid dropwise to the acetic anhydride at 0°. This solution was heated to 45° in a water-bath and 1.0 g. (0.0063 mole) of 2-nitramino-5-hydroxy-1,3-diazacyclohexene-2 was added with stirring. The solid dissolved and an exothermic reaction occurred during which considerable colorless gas was evolved from the reaction mixture. The reaction mixture was maintained at 45° for a period of three and a half hours after which it was cooled to 0°. At this temperature the product crystallized from the solution and crystallization appeared to be complete after four hours. The crystals were removed by filtration, washed thoroughly with water and dried. The crude product (m. p. 111–113°) weighed 1.0 g. (63.3%). Two crystallizations from glacial acetic acid raised the melting point to 112.3–112.7°. The analysis agrees with the theoretical values for 1,3-dinitro-5-nitroxy-1,3-diazacyclohexanone-2. In agreement with its structure this compound gives a positive Franchimont[6] test with both α-naphthylamine and N-dimethylaniline solutions.

Anal. Calcd. for $C_4H_6N_6O_8$: C, 19.1; H, 1.99; N, 27.9. Found: C, 19.3; H, 1.93; N, 27.8.

The nitric acid–acetic anhydride filtrate from the 1,3-dinitro-5-nitroxy-1,3-diazacyclohexanone-2 was poured onto 15 g. of ice. A white gum-like substance separated which changed to a white solid over a period of four days. This solid was filtered off and washed with water. A yield of 0.4 g. (28.2%) of crude 1,3-dinitramino-2-nitroxypropane (m. p. 152–158°) was obtained. After two recrystallizations from nitromethane, the melting point was raised to 160.4–160.5° dec.; yield 0.2 g. This compound gave a good Franchimont nitramine test[6] using α-naphthylamine.

Anal. Calcd. for $C_3H_7N_5O_7$: C, 16.0; H, 3.10; N, 31.1. Found: C, 16.1; H, 3.12; N, 30.6.

1,4-Dinitraminobutane.—1,4-Dinitraminobutane was prepared from 1,3-dinitro-1,3-diazacycloheptone-2 described by McKay and Wright.[1] It was hydrolyzed using

(6) A. P. N. Franchimont, *Rev. trav. chim.*, **16**, 226 (1897).

the procedure of Franchimont and Klobbie[3] for the preparation of 1,2-dinitraminoethane from 1,3-dinitro-1,-3-diazacyclopentanone-2. The crude yield of 1,4-dinitraminobutane (m. p. 159.6–160.5° with decomposition) was 91.8%. Two crystallizations from water (25 cc. per g.) raised the melting point to 162.2° dec. Dekkers[5] prepared this compound from N,N¹-dinitro-N,N¹-dicarbomethoxytetramethylenediamine and reported a melting point of 163°.

1,3-Dinitraminopropane.—1,3-Dinitraminopropane (m. p. 67.2–68°) was prepared in 93% yield from 1,3-dinitro-1,3-diazacyclohexanone-2[1] by the procedure described above for the preparation of 1,3-dinitraminobutane. Franchimont and Klobbie[4] have reported a melting point of 67° for 1,3-dinitraminopropane.

Summary

A series of nitration products has been prepared from 4(or 5)-methyl-2-nitramino-1,3-diazacyclopentene-2,4(or 6)-methyl-2-nitramino-1,3-diazacyclohexene-2, and 2-nitramino-5-hydroxyl-1,3-diazacyclohexene-2. The first compound was the only one that gave a 1-nitro derivative.

KINGSTON, ONTARIO RECEIVED NOVEMBER 15, 1948

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF WISCONSIN]

Properties of the Red Pigment from Soybean Nodules[1]

BY HENRY N. LITTLE[2]

A red pigment resembling animal hemoglobin has been observed in the nodules of leguminous plants. This pigment has been termed leghemoglobin by Virtanen, whereas Keilin and co-workers have applied the name hemoglobin. The more general term hemoprotein has been used in this paper. Kubo[3] found that the pigment was present in the nodules of all the leguminous plants he tested. Virtanen[4] reported that only plants with nodules containing visible amounts of the pigment actively fixed nitrogen; when the plants matured and the pigment disappeared from the nodules, nitrogen fixation ceased. Virtanen, *et al.*,[5] also observed a positive correlation between the hemin content of the nodules and the effectiveness of the bacterial strain used for inoculation. Keilin and Smith[6] concluded from the universal occurrence of the nodule "hemoglobin" in actively fixing nodules and the inhibition of nitrogen fixation by very low partial pressures of carbon monoxide that this pigment must be linked in some way to symbiotic nitrogen fixation.

The evidence to date concerning the nature of hemoprotein has been largely limited to the observation that the absorption peaks of this pigment and its derivatives agree well with those of the corresponding derivatives of animal hemoglobin. In addition Kubo[3] has reported that the form of the hemin crystals derived from nodule hemoprotein was similar to that from horse hemoglobin. Keilin and Wang,[7] and later Little and Burris,[8] observed that the oxygenated nodule hemoprotein could be deoxygenated by evacuation.

The nodule pigment has never been obtained in a crystalline state. Keilin and Wang[7] prepared pigment of 40–50% purity based on heme content. Virtanen[9] obtained pigment preparations having an iron and heme content corresponding to 80–85% purity.

The present paper reports a study of the purification of the pigment, its reversible splitting and reconstitution, and the nature of the porphyrin which it yields.

Experimental Methods

The nodule hemoprotein was prepared from soybean nodules by ammonium sulfate fractionation. After removal from the plant, the nodules were chilled and at all stages thereafter the preparation was maintained at 0–5°. The nodules were ground in approximately their own weight of *p*H 7.0, 0.1 *M* phosphate buffer; about 0.5 mg. of sodium hydrosulfite per gram of nodules was added and carbon monoxide was passed through the mixture during the grinding. The pulp was pressed by hand in cheesecloth. This extraction was repeated once or twice using one-half the original volume of buffer each time. Enough solid ammonium sulfate was added to the combined press juices to bring the solution to 1.9 *M*; the *p*H was adjusted to 7.0 and the solution centrifuged. The precipitate was discarded and to the supernatant solution was added enough solid ammonium sulfate to bring the concentration to 2.7 *M*; the *p*H was again adjusted to 7.0 and the solution centrifuged. The small amount of precipitate was discarded and the supernatant solution was made to 3.4 *M* ammonium sulfate. After standing one hour the solution was centrifuged. The resultant red precipitate was resuspended in water and dialyzed against numerous changes of distilled water. This pigment was used in further attempts at purification. In general about 1–2 mg. of crude nodule hemoprotein was obtained from each gram of fresh nodules.

The ratio of hemin content of the hemoprotein to dry weight, expressed as per cent. hemin, was used to determine changes of purity in different stages of purification. The hemin content was estimated by forming the pyridine hemochromogen, determining the optical density at 527 mμ with a Beckman spectrophotometer and comparing the absorption with a standard curve based on recrystallized hemin. To form the hemochromogen, 2 ml. of pyridine and 1 ml. of 3% ammonium hydroxide were added to 5

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(3) H. Kubo, *Acta Phytochim., Japan*, **11**, 195 (1939).

(4) A. I. Virtanen, *Biol. Rev.*, **22**, 239 (1947).

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(8) H. N. Little and R. H. Burris, THIS JOURNAL, **69**, 838 (1947).

(9) A. I. Virtanen, *Suomen Kemistilehti*, **19B**, 48 (1946)