The hydrogen uptake was complete in 3 min. Conventional workup afforded octahydroisoflavopereirine (13 mg.), m.p. 116–119° (from hexane).

(c).—A mixture of immonium salt IIIc (400 mg.), zinc dust (1 g.), acetic acid (5 ml.) and water (1 ml.) was refluxed for 1 hr. The initial yellow color had disappeared in 30 min. The mixture was filtered, the filtrate was concentrated and the residue basified with ammonium hydroxide and extracted with chloroform. The extractable material (290 mg.) was chromatographed on an alumina column (13 × 115 mm.). Benzene–hexane (1:2) elution afforded compound VIII (99 mg.), m.p. 116–119°, identical with the sample above. Continued elution with benzene yielded the isomer IX (112 mg., solvate from methanol). Drying and crystallization from hexane gave pure IX, m.p. 90–93°. Mixed m.p. and infrared spectra proved it to be identical with the sample prepared by cyclization of Ib.

Anal. Calcd. for $C_{17}H_{22}N_2$: C, 80.27; H, 8.72; N, 11.01. Found: C, 80.30; H, 8.67; N, 11.02.

Deuterated Octahydroisoflavopereirines VIII and IX.—The immonium perchlorate IIIc (198 mg.) was reduced with zinc dust as described in the preceding experiment. But acetic acid-*d* and deuterium oxide were substituted for acetic acid and water, respectively. The resulting amine mixture was refluxed for 20 min. in 0.1 *M* sodium *t*-butoxide in *t*-butyl alcohol (4 ml.) to ensure complete protonation at N_a, even though the infrared spectrum (chloroform) of the material prior to the base treatment did not indicate any isotope exchange during the reduction. The recovered material (148 mg.) was chromatographed on alumina to give VIII-3-*d* (53 mg.), m.p. 116–119° (from hexane), and IX-3-*d* (34 mg.), m.p. 90–93° (from hexane). Whereas no melting point depression was observed on admixture with the corresponding fully protonated compounds, the infrared spectra showed considerable differences throughout the fingerprint regions.

Tetrahydroisoflavopereirine Perchlorate (IV). (a).—A mixture of VIII (152 mg.), maleic acid (350 mg.) and palladium black (75 mg.) in water (10 ml.) was refluxed for 9 hr.[7] and then filtered while hot. After neutralization with sodium bicarbonate, excess sodium perchlorate was added to the filtrate. Crystallization of the precipitate from ethanol afforded tetrahydroisoflavopereirine perchlorate (167 mg.), m.p. 254–256.5°; ultraviolet spectrum (95% ethanol): λ_{max} 253 mμ(log ε 4.51), 307 mμ (log ε 4.37), 369 mμ(log ε 3.73); λ_{min} 226 mμ(log ε 4.14), 279 mμ(log ε 3.76), 327 mμ(log ε 3.20).

Anal. Calcd. for $C_{17}H_{19}O_4N_2Cl$: C, 58.20; H, 5.46; N, 7.99. Found: C, 58.16; H, 5.47; N, 7.91.

(b).—When the perchlorate IIIc (150 mg.) was dehydrogenated with maleic acid (175 mg.) and palladium black (75 mg.) following the procedure above, crystallization occurred after a few hours. After completed reaction, sodium perchlorate (100 mg.) was added to the neutralized mixture and the solid filtered off. The filter cake was triturated with 90% aqueous acetonitrile, the solution was concentrated and the crude product, obtained on cooling, crystallized from ethanol. This yielded tetrahydroisoflavopereirine perchlorate (120 mg.), m.p. 254–256°.

(c).—Dehydrogenation of compound VIII (51.5 mg.) by the method described below, precipitation with sodium perchlorate from a methanolic solution, afforded a crude product (50 mg.), m.p. 248–254°. Its ultraviolet spectrum was identical with that of compound IV except for a slightly higher absorption at 369 mμ (log ε 3.82). This would indicate that the dehydrogenation had stopped at the tetradehydro stage. Treatment with Norit in aqueous acetonitrile and repeated recrystallization from methanol yielded material identified as IV by m.p. 249–254.5°, mixed m.p. 250–255.5° and by its infrared spectrum (Nujol) which was superimposable on that of authentic material.

Flavopereirine Perchlorate (V).—Compound VI (9.5 mg.) was treated with hydrogen chloride in ethyl ether, the crude hydrochloride salt was mixed intimately with 10% palladium–charcoal (9 mg.) and the mixture heated at 275° for 10 min. The crude product was extracted with methanol. Its ultraviolet spectrum indicated that it was essentially a mixture of flavopereirine (V) and tetrahydroflavopereirine. Separation on a cellulose column as described previously[2] afforded a small quantity of a perchlorate, m.p. 221–226°, presumably the tetrahydro compound, as well as flavopereirine perchlorate (3 mg.), identified by its m.p. 326–330° (from water, stage preheated at 310°) and its infrared spectrum (KBr disk).[2,14]

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY, UNIVERSITY OF MINNESOTA, MINNEAPOLIS 14, MINN.]

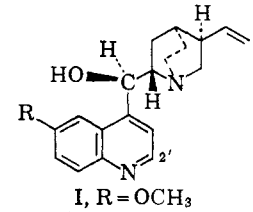
Biosynthesis of the Cinchona Alkaloids. I. The Incorporation of Tryptophan into Quinine[1]

BY NICHOLAS KOWANKO AND EDWARD LEETE

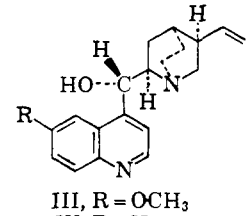
RECEIVED JULY 26, 1962

DL-Tryptophan-2-C^{14} was fed to one-year old *Cinchona succirubra* plants. Extraction of the plants six weeks later yielded radioactive quinine (0.7% incorporation) which was degraded systematically and found to have essentially all its activity located at C-2′ of its quinoline moiety. This result is in accord with a hypothesis which was suggested by Goutarel, Janot, Prelog and Taylor in 1950.

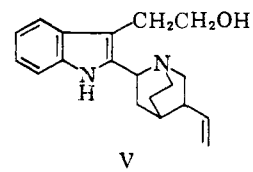
Quinine (I) is found in the bark of various *Cinchona* species,[2] and is usually accompanied by the related alkaloids cinchonidine (II), quinidine (III) and cinchonine (IV). Two of the minor alkaloids are cinchonamine (V) and quinamine (VI), and an examination of their structures led Goutarel, Janot, Prelog and Taylor[3] to suggest that all the Cinchona alkaloids have a common biogenetic origin. It was proposed that the quinoline moiety of the major alkaloids is formed from an indole having a two-carbon side chain at C-3



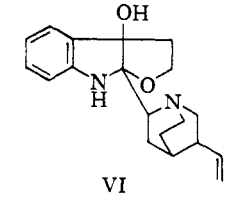
I, R = OCH_3
II, R = H



III, R = OCH_3
IV, R = H



V



VI

I, R = OCH_3
II, R = H

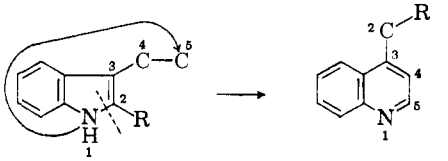
III, R = OCH_3
IV, R = H

(1) An account of this work was presented at the 142nd National Meeting of the American Chemical Society, Atlantic City, N. J., September 9–14, 1962. This investigation was supported by research grant MY-2662 from the National Institute of Mental Health, U. S. Public Health Service.

(2) *Cf.* J. J. Willaman and B. G. Schubert in "Alkaloid-Bearing Plants and Their Contained Alkaloids," Technical Bulletin No. 1234, U. S. Department of Agriculture, 1961, p. 189.

(3) R. Goutarel, M.-M. Janot, V. Prelog and W. I. Taylor, *Helv. Chim. Acta*, **33**, 150 (1950).

(VII). The bond cleavage and bond formation which are required to arrive at a quinoline nucleus are depicted schematically in Fig. 1. van Tamelen and Haarstad[4] have achieved such a transformation on a model system *in vitro*. The suggestion of Goutarel and co-workers that the indole derivative



VII

Fig. 1.

VII is derived from tryptophan[5] is a most reasonable one and we have tested their hypothesis by feeding tryptophan-2-C^{14} to one-year old *Cinchona succirubra*[6] plants. It has been previously shown that radioactive alkaloids were produced when specimens of this species of similar age were allowed to grow in an atmosphere containing carbon dioxide-C^{14}.[7] The plants were harvested six weeks after administration of the tryptophan to the stems by means of a cotton wick. Inactive quinine was added as a carrier to the crude plant extract since the yield of alkaloids was expected to be small. On reisolation and careful purification, quinine was obtained with a high specific activity (3.9×10^6 d.p.m./mM.) representing an incorporation[8] of at least 0.7%.

If tryptophan-2-C^{14}, or closely related metabolite, had been utilized in accordance with the biogenetic scheme illustrated in Fig. 1, activity would be expected at C-2′ in the quinine. The degradation illustrated in Fig. 2 was thus carried out to determine the activity at this position.

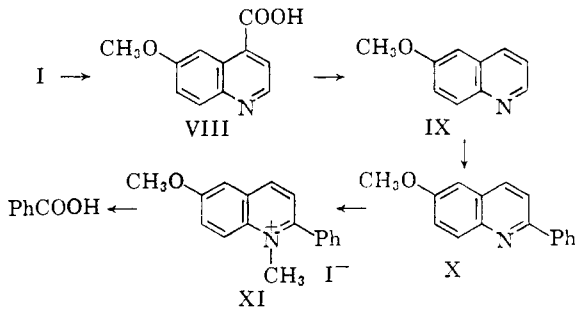


Fig. 2.—Method of degradation of the quinine-C^{14}.

Oxidation of the radioactive quinine with a mixture of chromium trioxide and manganese dioxide in sulfuric acid yielded quininic acid (VIII).[9] Decarboxylation on heating with an equal weight of copper chromite occurred smoothly at 250°, the evolved carbon dioxide being collected as barium carbonate which was found to have negligible activity. On raising the temperature of the reaction flask to 310°, 6-methoxyquinoline (IX) distilled, and was allowed to react with phenyllithium yielding 6-methoxy-2-phenylquinoline (X) which was identical with an authentic specimen obtained by the decarboxylation of 2-phenylquininic acid prepared from *p*-anisidine, benzaldehyde and pyruvic acid.[10] This compound was converted to its methiodide XI which was oxidized with alkaline potassium permanganate yielding benzoic acid which had essentially the same specific activity as the 6-methoxy-2-phenylquinoline. The results in Table I indicate that essentially all the radioactivity of the quinine was located at C-2′, and strongly support the hypothesis of Goutarel and co-workers concerning the biogenesis of the quinoline nucleus. Work is proceeding on the origin of the quinuclidine moiety of these alkaloids.

TABLE I

SPECIFIC ACTIVITIES OF QUININE AND ITS DEGRADATION PRODUCTS

	Activity,[11] d.p.m./mM. × 10^{-4}
Quinine sulfate $(B_2 \cdot H_2SO_4 \cdot 2H_2O)/2$	3.9
Quininic acid (VIII)	3.8
Barium carbonate (from quininic acid decarboxyln.)	<0.01
6-Methoxy-2-phenylquinoline (X)	3.7
Benzoic acid	3.5

Experimental[12]

Administration of DL-Tryptophan-2-C^{14} to Cinchona plants and Isolation of the Alkaloids.—The plants were grown from seed and were about 12 cm. high when they were one year old.[13] At this time three plants were threaded with a cotton wick and fed a solution of DL-tryptophan-2-C^{14}[14] (42.9 mg., 2.46×10^8 d.p.m./mM.). After 6 weeks the plants were harvested (wet wt. 45 g.) and macerated in a Waring Blendor with chloroform (600 ml.) and 15 *N* ammonia (200 ml.). Chromatographically pure quinine (140 mg., 174–175°) was added as a carrier and the mixture allowed to stand for 5 days. The mixture was then filtered through cloth yielding an aqueous layer (2.9×10^7 d.p.m.) and a chloroform layer which was concentrated to 100 ml. and extracted several times with 2 *N* hydrochloric acid. The acid extract was made basic with ammonia and extracted with chloroform. Evaporation of the dried chloroform solution yielded a brown gum having a total activity of 1.96×10^7 d.p.m.

A sample of the crude alkaloid was chromatographed on paper (Whatman No. 4) using cyclohexanol saturated with

(4) E. E. van Tamelen and V. B. Haarstad, *Tetrahedron Letters*, 390 (1961).

(5) Introduction of the methoxy group into quinine and quinidine is considered to occur at some stage in this biosynthesis.

(6) This species was used since it was the only one which we were able to cultivate in a greenhouse from seeds. We thank Dr. Robert F. Raffauf of Smith, Kline and French Laboratories for a generous supply of viable seeds.

(7) P. de Moerloose and R. Ruyssen, *J. Pharm. Belg.*, **8**, 156 (1953); *Pharm. Tijdschr. Belg.*, **30**, 97 (1953); P. de Moerloose, *Pharm. Weekblad.*, **89**, 541 (1954).

(8) Incorporation is defined as the total amount of activity in the isolated natural product divided by the amount of activity in the administered precursor. In this case it was calculated on the basis of the weight of inactive quinine added to the plant extract.

(9) H. John, *Ber.*, **63**, 2657 (1930).

(10) O. Döbner, *Ann.*, **249**, 98 (1888).

(11) Radioactivity measurements were carried out in a Nuclear Chicago model C-115 low background Q gas flow counter. With aluminum planchets the background was about 2.5 counts/min. Determinations were carried out on samples of finite thickness, making corrections for efficiency and self absorption.

(12) Melting points are corrected. Analyses were carried out by Mrs. Olga Hamerston and her assistants at the University of Minnesota.

(13) We are deeply grateful to Robert McLeester of the Botany Department of the University of Minnesota for the cultivation of the splendid specimens of *C. succirubra*.

(14) Purchased from TracerLab, Inc., Waltham, Mass.

3.5 *N* hydrochloric acid (10:3) as the developing solvent.[15] With this solvent the Cinchona alkaloids had the following R_f values: cinchonidine 0.52, cinchonine 0.56, quinine 0.58, quinidine 0.70. Radioactivity was detected on the paper at positions corresponding to these alkaloids. The crude alkaloid was dissolved in benzene and chromatographed on Woelm alumina (activity II–III). Elution was carried out with benzene followed by mixtures of benzene and chloroform. The fractions obtained by elution with a 1:1 mixture of benzene and chloroform contained quinine (detected by thin layer chromatography on basic silica gel). These fractions were combined (76 mg.) and crystallized twice from benzene, then from a mixture of benzene and petroleum ether. Finally the dried quinine was sublimed (160–165°, 0.001 mm.) affording 34.4 mg. of alkaloid, m.p. 174–175°. For degradation and further purification this quinine was diluted 25 times and crystallized from benzene. The diluted quinine (564 mg.) was dissolved in ethanol (10 ml.) and dilute sulfuric acid (4.25 ml. of a 2% solution). The solution was evaporated to dryness, water (25 ml.) added and boiled, adding two more drops of the 2% sulfuric acid. The hot solution was filtered and on cooling colorless needles of quinine sulfate ($B_2.H_2SO_4.2H_2O$) separated. After several crystallizations from hot water this material had a constant specific activity.

Oxidation of the Quinine sulfate-C^{14}.—Quinine sulfate (488 mg.) was dissolved in 10% sulfuric acid (10 ml.) and manganese dioxide (105 mg.) added. The mixture was raised to the boiling point and chromium trioxide (1.0 g.) dissolved in water (2 ml.) added during half an hour. The refluxing was continued for 3 hr. Hot water (90 ml.) and 15 *N* ammonia (20 ml.) were then added and after standing for 18 hr. on a steam-bath the mixture was filtered with the aid of Celite. The residue was extracted several times with hot dilute ammonia solution. The combined filtrates were evaporated to small bulk (15 ml.) and acidified with acetic acid yielding quininic acid (207 mg., 83%).

Decarboxylation of the Quininic Acid and Phenylation of the 6-Methoxyquinoline.—Dry quininic acid (205 mg.) was mixed with copper chromite catalyst[16] (205 mg.) and heated in a stream of carbon dioxide free nitrogen in a metal bath at 250–260° for 45 min. Carbon dioxide was liberated rapidly above 240° and was collected as barium carbonate by passing into 3% aqueous barium hydroxide solution. The metal bath temperature was then raised to 310° when 6-methoxyquinoline distilled as a pale yellow oil. This oil was washed out with toluene and dried over magnesium sulfate. The 6-methoxyquinoline dissolved in a 1:1 mixture of toluene and diethyl ether (5 ml.) was added at room temperature during 5 min. to a stirred solution of phenyllithium which had been prepared from lithium (22 mg.), bromobenzene (0.15 ml.) and ether (1 ml.). The yellow reaction mixture was then stirred at 80° for 5 hr. when a pale yellow solid separated. Finally the mixture was refluxed at 120° for 1 hr. After cooling, water was added and the organic layer extracted several times wih 2 *N* hydrochloric acid. The combined aqueous extracts were made basic with potassium hydroxide and extracted with chloroform. The dried chloroform on evaporation yielded a pale yellow solid which was sublimed *in vacuo* (120°, 0.001 mm.). Crystallization of the sublimate from aqueous ethanol yielded colorless plates of 6-methoxy-2-phenylquinoline (108 mg., 50%), m.p. 133–134°, not depressed on admixture with an authentic specimen.[10]

Anal. Calcd. for $C_{16}H_{13}NO$: C, 81.68; H, 5.57; N, 5.95. Found: C, 81.56; H, 5.81; N, 6.06.

Methylation and Oxidation of the 6-Methoxy-2-phenylquinoline.—6-Methoxy-2-phenylquinoline (51 mg.) was refluxed in methanol (20 ml.) with methyl iodide (3 ml.) in the presence of sodium carbonate (200 mg.) for 24 hr. Additional methyl iodide (1-ml. portions) were added 2, 8 and 16 hr. after the start of the reaction. The solvent was then removed and the residue dissolved in water (20 ml.) containing sodium carbonate (100 mg.). Potassium permanganate (3 g.) was added and the mixture refluxed for a few minutes and then allowed to cool slowly to room temperature with stirring. After 16 hr. the mixture was acidified with sulfuric acid and decolorized with sulfur dioxide. Extraction of the clear yellow solution with chloroform yielded benzoic acid which was purified by sublimation and crystallization from water (3.3 mg.); m.p. 121–122°, not depressed on admixture with an authentic specimen.

Activities of quinine and its degradation products are recorded in Table I, for the non-diluted material.

(15) D. J. Lussman, E. R. Kirch and G. L. Webster, *J. Am. Pharm. Assoc., Sci. Ed.*, **40**, 368 (1951).

(16) "Organic Syntheses," Coll. Vol. II, Ed. A. Blatt, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 142.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, CORNELL UNIVERSITY, ITHACA, N. Y.]

An Approach to the Specific Cleavage of Peptide Bonds. I. The Acyl Migration in Dipeptides Containing Hydroxyamino Acids in Anhydrous Hydrogen Fluoride

BY S. SAKAKIBARA,[1] K. H. SHIN AND G. P. HESS

RECEIVED MAY 29, 1962

The N,O-acyl migration in dipeptides containing serine or threonine was studied in anhydrous hydrogen fluoride. In some instances this reaction was also studied in concentrated sulfuric acid. All experiments were followed by Van Slyke amino-nitrogen determinations, paper electrophoresis, and isolation, crystallization, and identification of the reaction products by classical means. The analytical procedures were standardized with synthetic N- and O-acyl peptides. The experiments presented indicate that the N,O-acyl migration in dipeptides proceeds in anhydrous HF in high yields (90%) without unspecific cleavage of amide bonds or the formation of significant amounts of side products. In contrast to this are the results obtained in sulfuric acid. In the case of glycyl-DL-serine, a maximum yield of 35% of the desired O-peptide was obtained and a great number of side products could be detected.

In view of the outstanding importance of obtaining amino acid sequences of proteins and the complex problems associated with the specific degradation of these molecules to small peptides amenable to structure determination, we have explored a method for the specific cleavage of peptide bonds using anhydrous hydrogen fluoride.

The reversible, *p*H dependent, N,O-acyl migration of β-amino alcohols was first investigated by Bergman[2a] in 1923; later studies[2b] suggested that a hydroxyoxazolidine (I) is the intermediate in this reaction.

Although in strong acids this reaction has previously been investigated with only two model peptides,[3] both containing serine, a great number of

(1) Institute for Protein Research, Osaka University, Osaka, Japan.

(2) (a) M. Bergmann, E. Brand and F. Weinmann, *Hoppe-Seyler's Z. Physiol. Chem.*, **131**, 1 (1923); (b) A. P. Phillips and R. Baltzly, *J. Am. Chem. Soc.*, **69**, 200 (1947); W. E. Hanby, S. G. Waley and J. Watson, *J. Chem. Soc.*, 3239 (1950).

(3) J. A. Moore, J. R. Dice, E. D. Nicolaides, R. D. Westland and E. L. Wittle, *J. Am. Chem. Soc.*, **76**, 2884 (1954); H. Hoermann, W. Grassman, E. Wuensch and H. Preller, *Ber.*, **89**, 933 (1956).