ferricyanide while 1 *N* NH_4OH was added to maintain a pH of 7. An excess of 0.01 *N* potassium ferricyanide solution (5 ml) was then added and a yellow color persisted for at least 20 min. The solution was stirred with 35 ml (wet volume) of the chloride form of AG3X4 resin (Bio-Rad Laboratorties, Richmond, Calif.) for 15 min to remove ferrocyanide and excess ferricyanide ions. The oxidation and ion-exchange procedures were carried out within a period of 1 hr at 25°. The solution obtained after removal of the resin by filtration was lyophilized.

The crude product was subjected to partition chromatography on Sephadex G-25 at 25° under the following conditions: solvent, 1-butanol–pyridine–0.2 *N* acetic acid (6:1:7); column size, 2.85 × 58.0 cm; hold-up volume, 78.7 ml; flow rate, 25 ml/hr; fraction size, 9.2 ml; aliquots for Folin–Lowry determinations, 0.10 ml; solvent for regeneration of column, pyridine–0.1% acetic acid (1:4). One major peak was detected in the chromatogram with an R_f value of 0.15. Partition chromatography of an authentic sample (2 mg) of acetone-lysine-vasopressin under the same conditions gave a peak with R_f 0.16. The eluates represented by the peak with R_f 0.15 were mixed with 360 ml of ice-cold water, evaporated *in vacuo* to low volume in a bath at 15°, and lyophilized, yield 58 mg. This product was rechromatographed on Sephadex G-25 at 4° under the following conditions: solvent, 1-butanol–ethanol–pyridine–0.2 *N* acetic acid (14:2:5:24); column size, 2.20 × 52 cm; hold-up volume, 49.3 ml; flow rate, 12 ml/hr; fraction size, 3.3 ml; aliquots for Folin–Lowry determination, 0.05 ml; solvent for regeneration of column, pyridine–0.1% acetic acid (1:4). One peak was detected with R_f 0.36, and the material isolated from the eluates represented by the central portion of the peak weighed 42 mg. Chromatography of an authentic sample of acetone-lysine-vasopressin under the same conditions gave a peak with R_f 0.36.

Comparisons of Synthetic and Authentic Acetone-lysine-vasopressin. A sample (1 mg) of the synthetic material dissolved in 0.05 ml of 0.1% acetic acid gave a negative Legal test for acetone, but after the solution was heated at 90° for 15 min a positive test was obtained. A quantitative determination showed that 89% of the theoretical amount of acetone had been liberated from the compound. The results in these two tests were the same, within experimental error, as those obtained with an authentic sample of acetone-lysine-vasopressin.[3] The synthetic preparation (1.2 mg) was subjected to gel filtration on Sephadex G-25 as follows: solvent, 0.2 *N* acetic acid at 25°; column size, 1.38 × 83.5 cm; flow rate, 7 ml/hr; fraction size, 0.79 ml; aliquots for Folin–Lowry determination, 0.79 ml. Only one peak, with a maximum at effluent volume 102 ml, was detected. This is the same effluent volume at which the maximum occurred on gel filtration of authentic acetone-lysine-vasopressin.

The synthetic material exhibited the specific rotation $[\alpha]^{21}D$ −72.4° (*c* 0.5, 0.1 *N* acetic acid in 67% aqueous acetone), as compared to $[\alpha]^{17}D$ −74° (*c* 0.5, 0.1 *N* acetic acid in 67% aqueous acetone) reported for acetone-lysine-vasopressin.[3] The synthetic material has a pressor activity of 6–7 units/mg, the same as that previously reported for acetone-lysine-vasopressin.[3]

Acknowledgments. The authors wish to thank Miss Margitta Wahrenburg and Mrs. Jessie Lawrence, who performed the rat pressor assays under the direction of Dr. W. Y. Chan, and Dr. Donald Yamashiro, who kindly made available the isopropylidene-S-benzyl-L-cysteinyl-L-tyrosine used in this investigation.

Biosynthesis of the *Cinchona* Alkaloids. II. The Incorporation of Tryptophan-1-^{15}N,2-^{14}C and Geraniol-3-^{14}C into Quinine[1]

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Abstract: The administration of DL-tryptophan-1-^{15}N,2-^{14}C (prepared from indole-1-^{15}N,2-^{14}C) to *Cinchona succirubra* plants yielded labeled quinine having all its ^{14}C at C-9, and excess ^{15}N only on the quinoline nitrogen. Furthermore, the specific incorporation of ^{14}C and ^{15}N into these positions was identical (0.97%). These results strongly support the hypothesis of Goutarel, Janot, Prelog, and Taylor, who suggested in 1950 that quinine and related quinoline alkaloids found in *Cinchona* species are derived from indole alkaloids of the corynantheine type. Additional evidence favoring this biosynthetic scheme was obtained by feeding geraniol-3-^{14}C to the same species, when quinine labeled specifically at C-10 of the quinuclidine nucleus was obtained. This result is in agreement with recent work on the origin of the nontryptophan-derived portion of indole alkaloids containing an extra nine- or ten-carbon unit.

We have previously shown[3] that the administration of tryptophan-α-^{14}C (label in the alanyl side chain) to *Cinchona succirubra* plants yielded quinine (**9**) which had essentially all its activity located at C-2′ of its quinoline moiety, This result was in accord with the biosynthetic scheme illustrated in Scheme I, which is an elaboration of the one suggested by Goutarel, *et al.*,[4] in 1950. It was proposed that quinine and related *Cinchona* alkaloids are formed from an indole derivative such as **6**, which is plausibly formed by a Mannich reaction between tryptophan (**1**) and the ten-carbon unit **2**, the origin of which will be discussed later. The alkaloid corynantheine has the carbon skeleton of compound **6**. Cleavage of the tetrahydro-β-carboline ring, loss of the carboxyl group, and formation of the quinu-

(1) This investigation was supported by Research Grant GM-13246 from the U. S. Public Health Service. A preliminary account of part of this work has appeared as a communication: E. Leete and J. N. Wemple, *J. Am. Chem. Soc.*, **88**, 4743 (1966).

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Scheme I. Hypothetical Biosynthetic Scheme for Quinine

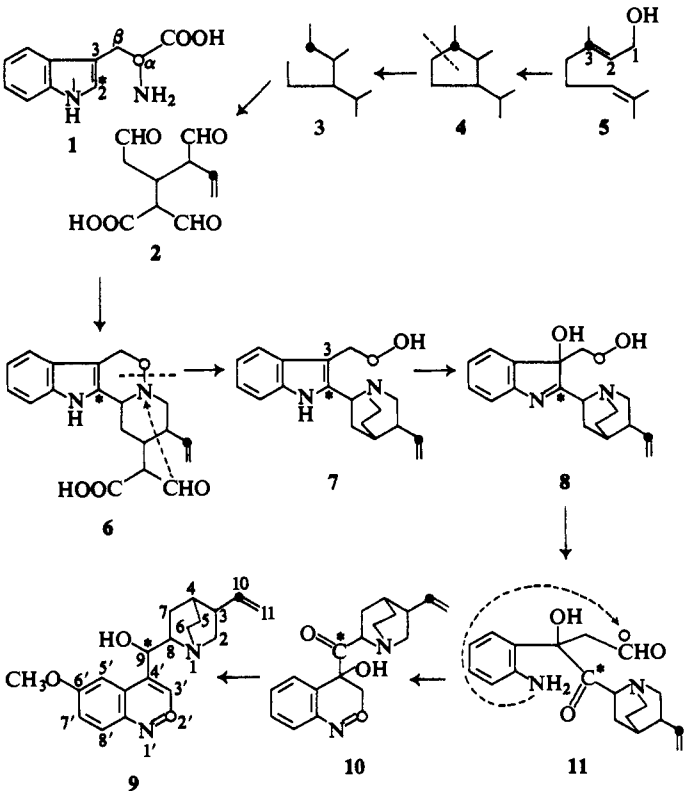


Table I. Activity of Quinine and Its Degradation Products

	Percentage excess ^{15}N	Spec act., dpm/mmol
Precursor fed: DL-Tryptophan-1-^{15}N,2-^{14}C	89.6[a]	9.07×10^7
Quinine		8.8×10^5
Dihydroquinine sulfate ($C_{20}H_{26}N_2O_4 \cdot 0.5H_2SO_4 \cdot H_2O$)	0.434[b]	8.8×10^5
Quininic acid	0.848	8.6×10^5
Barium carbonate[c]		8.4×10^5
Specific incorporation $^{15}N = 2 \times 0.434/89.6 = 0.97\%$		
Specific incorporation $^{14}C = \frac{8.8 \times 10^5}{9.07 \times 10^7} = 0.97\%$		
Precursor fed: Geraniol-3-^{14}C		1.45×10^9
Quinine		8.1×10^4
Dihydroquinine		7.6×10^4
1-Acetamidonaphthalene		7.5×10^4
1-Propionamidonaphthalene		7.6×10^4
Barium carbonate[d]		7.4×10^4
Specific incorporation $^{14}C = \frac{8.1 \times 10^4}{1.45 \times 10^9} = 0.006\%$		

[a] Present on the indole nitrogen. [b] Average of the excess ^{15}N on the quinoline and quinuclidine nuclei. [c] The specific activity of 8.4×10^5 is the average activity of the barium carbonate obtained from two separate degradations starting with dihydroquinine. [d] Obtained by a Schmidt reaction on the acetic acid produced by Kuhn–Roth oxidation of dihydroquinine.

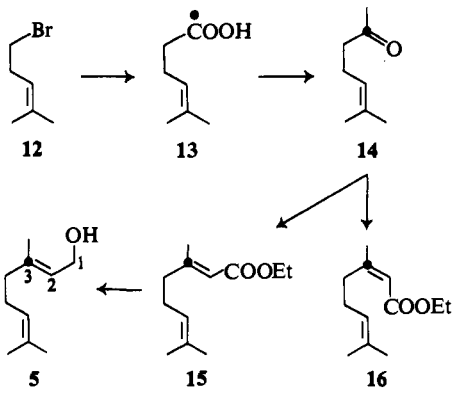
clidine ring affords cinchonamine (**7**), which is one of the minor alkaloids of *Cinchona* bark. Oxidation of cinchonamine by electrophilic attack at C-3 with ^+OH could afford the 3-hydroxyindolenine derivative **8**.[5] Opening of the indolenine ring and oxidation of the primary alcohol group yields compound **11**, which on cyclization affords **10**. Further steps to quinine, involving dehydration, reduction, hydroxylation of the quinoline nucleus, and methylation are unexceptional. The progress of the α-carbon of tryptophan through this sequence is indicated by means of open circles. In order to further substantiate this hypothesis we have now carried out a feeding experiment with tryptophan labeled with ^{14}C at C-2 of its indole nucleus, and with ^{15}N on the indole nitrogen. The labeled tryptophan was prepared as follows. Ammonium chloride-^{15}N and *o*-toluyl chloride in the presence of base yielded *o*-tolu-

(5) B. Witkop, *J. Am. Chem. Soc.*, **72**, 2311 (1950), has achieved this oxidation *in vitro* with peracetic acid.

amide-^{15}N, which was subjected to a Hofmann rearrangement with sodium hypobromite affording *o*-toluidine-^{15}N. This was formylated with formic acid-^{14}C yielding N-formyl-*o*-toluidine. Cyclization with potassium tertiary butoxide yielded indole-1-^{15}N,2-^{14}C.[6] This labeled indole was converted to tryptophan by established methods.[7] The quinine obtained from plants which had been fed this doubly labeled tryptophan contained both ^{14}C and excess ^{15}N, the specific incorporation[8] of the ^{14}C being identical with that of the ^{15}N (0.97%), if it was assumed that all the excess ^{15}N was located on the quinoline nitrogen. Degradations showed that this was indeed the case. Oxidation of 10,11-dihydroquinine yielded quininic acid (6-methoxyquinoline-4-carboxylic acid) which had twice the percentage excess ^{15}N of that found in the dihydroquinine (Table I) indicating that there was negligible excess ^{15}N on the quinuclidine nitrogen. The nitrogen-15 determinations were carried out using a modification of the method of Günther, Floss, and Simon.[9] All the ^{14}C was shown to be present at C-9 by decarboxylation of the quininic acid, the liberated carbon dioxide having essentially the same specific activity as the quinine (Table I).

According to the biosynthetic scheme in Scheme I, the vinylquinuclidine moiety of quinine is derived from the aldehyde **2**. This origin of this hypothetical ten-carbon unit became apparent from related work on the biosynthesis of the indole alkaloids of *Vinca rosea* which had been carried out by Scott,[10] Arigoni,[11] Battersby,[12] and ourselves.[13] The nontryptophan-derived portion of the *Vinca* alkaloids, ajmalicine, vindoline, and catharanthine, was shown to be derived from geraniol. These results confirmed a hypothesis which had been formulated independently in 1961 by Wenkert[14] and Thomas,[15] and is depicted schematically in Scheme I. They suggested that the branched ten-carbon unit **3**, found in corynantheine and related alkaloids, is formed by cleavage of a cyclopentanomonoterpene **4**, which could be formed from the acyclic terpene geraniol (**5**). We therefore fed geraniol-3-^{14}C to *Cinchona* plants and radioactive quinine was indeed produced. The expected location of activity was on the vinyl group at C-10, and degradation of the quinine confirmed this. A Kuhn–Roth oxidation of dihydroquinine yielded a mixture of acetic and propionic acids. A Schmidt reaction on the acetic acid yielded carbon dioxide which had essentially the same specific activity as the quinine. Our work was complimented by an independent investigation of Battersby and coworkers,[16] who fed geraniol-2-^{14}C to *Cinchona ledgeriana* plants and obtained quinine labeled specifically at C-3 of the quinuclidine moiety. The geraniol-3-^{14}C was synthesized by the route illustrated in Scheme II. 5-Methyl-4-hexenoic acid-1-^{14}C (**13**) was obtained by reaction of the Grignard reagent made from 5-bromo-2-methyl-2-pentene (**12**) with carbon dioxide-^{14}C. Reaction of **13** with thionyl chloride produced the acid chloride which was treated with methylzinc iodide to yield 6-methyl-5-hepten-2-one-2-^{14}C (**14**). Reaction of this ketone with triethylphosphonoacetate in the presence of sodium hydride yielded a mixture of *cis*- and *trans*-ethyl 3,7-dimethyl-2,6-octadienoate-3-^{14}C (**16** and **15**, respectively) which were separated by preparative thin layer chromatography. Reduction of the *trans* isomer with lithium aluminum hydride yielded geraniol-3-^{14}C (**5**).

Scheme II. Synthesis of Geraniol-3-^{14}C



There have been speculations on the intermediates between geraniol and the ultimate *Corynanthe* unit (**3**) found in alkaloids of the corynantheine type.[17] Loganin is apparently an important intermediate *en route* to the indole alkaloids of *Vinca rosea*,[18,19] and it will be of interest to discover whether this compound is involved in the biosynthesis of quinine. Work is in progress.

Experimental Section

Melting points are corrected. Radioactivity measurements were carried out in a Nuclear Chicago Model 724 liquid scintillation system using as solvents either toluene or dioxane–water, with the usual scintillators.[20]

DL-**Tryptophan-1-^{15}N,2-^{14}C.** Freshly distilled *o*-toluyl chloride (1.90 g, 12.3 mmol) was added to a stirred cooled mixture of chloroform (125 ml), 5% aqueous sodium hydroxide (20 ml), and ammonium chloride-^{15}N[21] (0.67 g, 12.3 mmol, 95% excess ^{15}N). After stirring for 1 hr at 0°, the mixture was allowed to warm up to room temperature and the chloroform layer separated. The aqueous layer was extracted with additional chloroform, and the combined extracts were dried over sodium sulfate. Evaporation and crystallization of the residue from a mixture of benzene and chloroform yielded colorless needles of *o*-toluamide-^{15}N (0.81 g, 48%), mp 145–146°. Bromine (0.45 ml, 8.3 mmol) was slowly added to a stirred solution of *o*-toluamide-^{15}N (0.78 g, 5.73 mmol) and sodium methoxide (0.73 g, 6.18 mmol) in methanol (40 ml) at 0°, until the

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(8) Specific incorporation is defined as the dpm/mmol, or percentage excess ^{15}N found in the quinine, divided by the dpm/mmol, or percentage excess of ^{15}N present in the administered tryptophan.
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(17) A. R. Battersby, *Pure Appl. Chem.*, **14**, 117 (1967).
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(20) A. R. Friedman and E. Leete, *J. Am. Chem. Soc.*, **85**, 2141 (1963).
(21) Purchased from Tracerlab, Waltham, Mass.

solution remained permanently yellow. The reaction mixture was then refluxed for 1 hr. The residue obtained on evaporation of the methanol was dissolved in 20% sodium hydroxide (100 ml) and refluxed for 2 hr. After cooling the mixture was extracted with ether. Analysis of the crude extract by gas chromatography indicated that the *o*-toluidine was contaminated with about 5% of *p*-bromo-*o*-toluidine, which was converted to *o*-toluidine by hydrogenation as follows. The residue obtained on evaporation of the ether extract was dissolved in 85% ethanol containing sodium hydroxide (1 g), and hydrogenated in the presence of Raney nickel (0.1 g) at 2 atm pressure for 18 hr. The filtered reaction mixture was acidified with hydrochloric acid and evaporated to dryness. The residue was dissolved in water, made basic with sodium hydroxide, and extracted with ether. The ether was extracted with 10% hydrochloric acid which was evaporated to dryness yielding *o*-toluidine-^{15}N hydrochloride (0.72 g, 87%). Sublimation at 80–90° (1 mm) yielded colorless needles, mp 213–214°. The *o*-toluidine-^{15}N hydrochloride (1.20 g) was dissolved in water, made basic with ammonia, and extracted with ether. The dried (NaOH) ether extract was evaporated and the residual oil heated with 97% formic acid (0.4 ml) and sodium formate-^{14}C[21] (16 mg, 1 mCi) at 100° for 1 hr. More formic acid (0.2 ml) was then added and the heating continued for another hour. After cooling, the reaction mixture was diluted with 10% acetic acid (5 ml) and extracted several times with ether. Evaporation of the dried (Na_2SO_4) extract yielded a brown oil which was distilled at 70–80° (0.01 mm) affording N-formyl-^{14}C-*o*-toluidine-^{15}N (1.02 g, 90%), mp 57–58° (lit.[6] 61°). This compound was converted to indole and gramine by previously described methods.[22] Gramine-1-^{15}N,2-^{14}C (235 mg) and ethyl acetamidocyanoacetate (229 mg) were added to a rapidly stirred mixture of powdered sodium hydroxide (25 mg) and refluxing toluene (15 ml). After 9 hr the mixture was cooled and ethyl α-acetamido-α-cyano-β-(3-indole)-propionate (360 mg) separated. This material was refluxed with 15% sodium hydroxide (2.5 ml) for 10 hr and then filtered and the filtrate adjusted to a pH of 2.5 with hydrochloric acid. The solution was added to a column of Dowex 50W-X8 resin (20 g, H^+ form). The column was washed with water (200 ml) and then the tryptophan eluted with 1 *N* ammonia. Evaporation of the fractions which absorbed in the ultraviolet at 280 mμ (the indole absorption) yielded a white residue which was crystallized from aqueous ethanol affording colorless plates of DL-tryptophan-1-^{15}N,2-^{14}C (133 mg, 48%), mp 282–284° dec (lit.[23] 275–282° dec).

Geraniol-3-^{14}C. 5-Methyl-4-hexenoic Acid-1-^{14}C. 5-Bromo-2-methyl-2-pentane[24] (0.8 ml) was added to magnesium (0.12 g) suspended in dry ether (10 ml) and stirred with a magnetic stirrer. After 25 min, all the magnesium had dissolved, and the contents of the flask were frozen in liquid nitrogen. Carbon dioxide-^{14}C was generated from barium carbonate (0.98 g, 3 mCi) with concentrated sulfuric acid and condensed in the reaction flask. After warming to room temperature and stirring for 1 hr, the reaction mixture was acidified with 2 *N* hydrochloric acid and extracted with ether (three 100-ml portions). The ether was then extracted with 10% sodium carbonate (two 50-ml portions) which was then acidified with phosphoric acid and extracted with ether. Evaporation of the dried (Na_2SO_4) extract yielded 5-methyl-4-hexenoic acid-1-^{14}C (0.52 g, 81%).

6-Methyl-5-hepten-2-one-2-^{14}C. The hexenoic acid (300 mg) was dissolved in ether (10 ml) and a drop of pyridine and thionyl chloride (1 ml) added. After standing at room temperature for 1 hr, the solvent was removed, benzene added to the residue, and the evaporation repeated, yielding 5-methyl-4-hexenoyl chloride. Magnesium (0.125 g) was suspended in dry ether (10 ml), and excess methyl iodide (2 ml) added. When all the magnesium had dissolved, freshly fused zinc chloride (0.6 g) dissolved in ether (5 ml) was added and the mixture stirred for 10 min. The hexenoyl chloride, dissolved in benzene (5 ml), was added to the solution of methylzinc iodide stirred at 0°. After 2 hr the mixture was acidified with 2 *N* sulfuric acid (10 ml) and extracted with ether (50 ml), which was then washed with 10% sodium carbonate solution. The oil obtained on evaporation of the ether solution was shaken with a solution of semicarbazide hydrochloride (0.5 g) and sodium acetate hydrate (0.75 g) in water (10 ml). A copious white crystalline precipitate of the semicarbazone of 6-methyl-5-hepten-2-one-2-^{14}C (190 mg, 45%) separated. Crystallization from a mixture of ethyl acetate and petroleum ether yielded colorless plates, mp 135–136° (lit.[25] 135–136°), having an activity of 8.1×10^6 dpm/mg (1.47×10^9 dpm/mmol).

***cis*- and *trans*-Ethyl 3,7-Dimethyl-2,6-octadienoates-3-^{14}C.** The previously described semicarbazone (142 mg) was added to 0.5 *N* sulfuric acid (20 ml) and the mixture distilled. The distillate was extracted with ether and dried over magnesium sulfate. The residue obtained on evaporation was dissolved in 1,2-dimethoxyethane (2 ml) and added to a solution of triethylphosphonoacetate[26] (0.448 g) in dimethoxyethane (3 ml) which had previously been cooled and allowed to react with sodium hydride (0.1 g of a 54% suspension in oil). The mixture was stirred at room temperature for 24 hr and then warmed to 50° for 1 hr. Water was then added to the cooled reaction mixture, which was then extracted with ether. The oil obtained on evaporation of the dried ($MgSO_4$) extract was subjected to thin layer chromatography on a preparative plate of Silica Gel F_{254} (Merck). The plate was developed three times with a 1:1 mixture of benzene and petroleum ether (bp 60–70°), the *cis* ester having a higher R_f than the *trans* isomer. The zones were eluted with 20% methanol in methylene chloride. Evaporation of the dried ($MgSO_4$) eluates yielded *cis*-ethyl 3,7-dimethyl-2,6-octadienoate-3-^{14}C (22 mg) and the *trans* isomer (87 mg) (72% total yield).

Geraniol-3-^{14}C. *trans*-Ethyl 3,7-dimethyl-2,6-octadienoate-3-^{14}C (80 mg) was dissolved in ether (10 ml) and cooled to −80°. A 4% solution of lithium aluminum hydride in ether (5 ml) was added and the mixture stirred at −80° for 1 hr. The solution was allowed to warm to room temperature and ethyl acetate cautiously added to decompose the excess hydride. Aqueous 10% sodium carbonate was then added and the mixture extracted with ether. Evaporation of the dried ($MgSO_4$) extract yielded geraniol-3-^{14}C (49 mg, 80%), having an activity of 9.5×10^6 dpm/mg (1.45×10^9 dpm/mmol). This material was identical (infrared spectrum, thin layer chromatography, gas chromatography) with authentic geraniol.

Administration of Labeled Compounds to *Cinchona succirubra* and Isolation of the Quinine. *Cinchona* plants about 30 cm in height were obtained from an 8-year-old plant by air layering the stems of this plant.[27] The plants were fed about 6 months after the rooted stems had been separated from the parent plant. DL-Tryptophan-1-^{15}N,2-^{14}C (60 mg, 44.8% excess ^{15}N, total ^{14}C: 2.66×10^7 dpm) was dissolved in 0.1 *N* acetic acid (10 ml) and fed to two plants during a period of 7 days, by means of cotton wicks inserted into the stems. After 3 weeks the plants were harvested (fresh weight 120 g) and extracted as previously described,[3] except that no carrier quinine was added. Chromatography of the crude alkaloids (350 mg) on neutral Woelm alumina (activity II, 40 g) yielded, on elution with chloroform, initial fractions containing cinchonamine and other indole alkaloids. Later fractions contained quinine mixed with other quinoline alkaloids. Preparative thin layer chromatography[28] was carried out on Silica Gel PF_{254} plates (Merck) developing with a mixture of cyclohexanol, cyclohexane, petroleum ether (bp 60–70°), and diethylamine (6:6:6:1). With this system the following alkaloids could be separated, having the R_f values indicated in parentheses: quinine (0.35), cinchonidine (0.43), cinchonine and quinidine (0.50–0.60). The quinine was further purified by thin layer chromatography on Alumina PF plates (Merck), developing with a mixture of benzene, chloroform, and diethylamine (6:12:1), an R_f value of 0.45 being observed for quinine. The alkaloids were detected by their fluorescence in ultraviolet light. After several purifications by thin layer chromatography quinine (14.9 mg), mp 174–175°, was obtained with a constant specific activity (8.82×10^5 dpm/mmol). The ultraviolet spectrum of this purified quinine was essentially identical with literature data:[29] λ_{max}^{EtOH} mμ (log ε), 231 (4.52), 280 (3.61), 320 (3.64), and 332 (3.72). The optical rotation was a better criterion of purity since the other *Cinchona* alkaloids have quite different

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(27) We thank Mr. Bradford W. Gregory of the Botany Department of the University of Minnesota for propagating the splendid specimens of *Cinchona succirubra*.
(28) W. Kamp, W. J. M. Onderberg, and W. A. van Seters, *Pharm. Weekblad.*, **98**, 993 (1963).
(29) J. Holubek and O. Strouf, "Spectral Data and Physical Constants of Alkaloids," Vol. II, Heyden and Sons Ltd., London, 1966, p 224.

specific rotations: $[\alpha]^{20}D$ −159° (absolute EtOH) (lit.[29] −158°). Geraniol-3-^{14}C (17.6 mg, 1.67×10^8 dpm) was emulsified in water (30 ml) with the aid of Tween 80 (0.05 ml) and then fed to three *C. succirubra* plants by means of cotton wicks inserted in the stems. The plants were harvested 3 weeks later and yielded purified quinine having an activity of 8.1×10^4 dpm/mmol.

Degradation of the Quinine. When degradations were carried out to determine the location of the ^{14}C, considerable dilutions with inactive quinine could be tolerated; however, for the ^{15}N determinations the dilution was kept to a minimum, so that the excess ^{15}N in the labeled degradation products could be kept as high as possible above the natural abundance of ^{15}N (0.37%).

10,11-Dihydroquinine. Quinine (29.6 mg) was dissolved in ethanol (10 ml) and hydrogenated in the presence of Adams catalyst (6 mg) at room temperature and atmospheric pressure for 2 hr. Evaporation of the filtered mixture yielded a residue which was sublimed at 140° (0.001 mm) yielding dihydroquinine (26.2 mg, 88%), mp 171–172° (lit.[30] 172°). The sulfate was formed by addition of the calculated amount of sulfuric acid to a solution of dihydroquinine in ethanol, and then evaporating the solvent.[31]

Quininic Acid. Dihydroquinine sulfate (27.5 mg) and manganese dioxide (7.6 mg) were added to 8% sulfuric acid (1 ml), and the mixture was refluxed. Chromium trioxide (61 mg) was added during 30 min, and the mixture then refluxed for 3 hr. Ammonia (2 *N*, 10 ml) was added, and after heating on a steam bath for an additional 3 hr the mixture was filtered, the residue being washed with additional dilute ammonia. The combined filtrates were evaporated to dryness, redissolved in a little water, and acidified with acetic acid when quininic acid separated (4.1 mg, 31%), mp 282–284° dec. The quininic acid was decarboxylated as previously described.[3]

Kuhn–Roth Oxidation of Dihydroquinine. Dihydroquinine (150 mg) was dissolved in 2 *N* sulfuric acid (10 ml) and chromium trioxide (5 g) added. The mixture was distilled, water being added to maintain the volume in the reaction flask between 10 and 15 ml. The distillate was neutralized with 0.1 *N* sodium hydroxide (4.5 ml) and evaporated. Analysis of the residue by paper chromatography[32] indicated the presence of sodium acetate and sodium propionate. These were separated by partition chromatography on silicic acid[33] yielding sodium acetate (21 mg) and sodium propionate (9 mg). Portions of these salts were converted to 1-acetamidonaphthalene and 1-propionamidonaphthalene by previously described methods.[34] A Schmidt reaction[20] on the sodium acetate yielded barium carbonate, which was assayed by decomposition with concentrated sulfuric acid, collecting the liberated carbon dioxide in Hyamine 10-X (Rohm and Haas) dissolved in toluene.

^{15}N Determinations. The following method is a modification of that described by Günther, *et al.*[9] We thank Mr. Adrian Swanson of the Mass Spectrometry Laboratory of the University of Minnesota for invaluable help in developing this experimental procedure for analyzing less than 1 mg of an organic nitrogen compound. Calcium oxide (25 mg) and cupric oxide (25 mg) were heated in a Pyrex tube (10 cm long × 8 mm diameter) sealed at one end, at 650–700° for 3–4 hr in an oven. The tube was allowed to cool and the organic nitrogen compound (0.5 mg) added. Two constrictions were then made in the tube, one about 5 cm from the closed end, and the other near the open end. The tube was then attached to a high-vacuum system (10^{-3} to 10^{-4} mm) and after evacuation for 10 min was sealed at the outer constriction. The tube was heated at 650–700° in an oven for 90 min. After cooling to room temperature the tube was scratched carefully with a file at the remaining constriction. The tube was then placed in a larger tube attached by means of a vacuum line to an Hitachi-Perkin-Elmer RMU-6D mass spectrometer. This larger tube had a ball joint about 5 cm from its closed end, and by bending this tube at the ball joint the reaction tube was broken and the nitrogen gas from the Dumas decomposition passed to the mass spectrometer, the peak heights at 28 and 29 mass numbers being measured. Several determinations were carried out on each compound analysed and results having a standard deviation of less than 1% were obtained. Compounds to be analyzed were diluted with unenriched material so that the excess ^{15}N was 0.2–0.4%.

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