The Synthesis of ^{14}C-Labeled Chlorogenic Acid

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SUMMARY

^{14}C-labeled chlorogenic acid (specific activity 30.6 nCi/mg) was synthesized by reaction of diphenylmethyl-1-0-ethoxy-carbonyl-4,5-0-isopropylidenequinate (V) with 0,0-dimethylcarbonyl caffeoyl chloride-α-^{14}C (VIII) followed by selective hydrolysis of the protecting groups.

INTRODUCTION AND RESULTS.

Chlorogenic acid (3-0-caffeoylquinic acid) is widely distributed in many plants [1]. Its occurrence in tobacco is reported to vary between one to three percent, depending on the age and variety of plant at harvest [2]. During a study in our laboratory the need arose to synthesize specifically labeled chlorogenic acid.

Panizzi and co-workers [3a, b] and Ishizaka and Kouno [4] have reported a synthesis of chlorogenic acid. We were unable to duplicate their results without modifying the published procedures. Chart 1 shows the successful pathway used to obtain ^{14}C-labeled chlorogenic acid.

Quinic acid (I) was converted to the quinide (II) in good yield, following the method of Hanson [5]. The carbonate ester (III) was prepared from II by reaction with ethyl chloroformate. Opening of the lactone ring in III while retaining the carbonate ester group proved to be very difficult. Several conditions reported by Hanson [5] and by Panizzi [3b] were tried without success. Usually a secondary reaction occurred to produce methyl-1-0-ethoxycarbonyl-4, 5-0-isopropylidene quinate (IV*b*). The compound was difficult to purify and reverted to III on vacuum distillation. Treatment of III with aqueous sodium hydroxide in warm acetone gave the hydroxyacid (IV*a*) in good yield. The carboxylic acid group was protected by transformation to the diphenylmethyl derivative. This completed the sequence of protecting quinic acid for subsequent reaction with radioactive material.

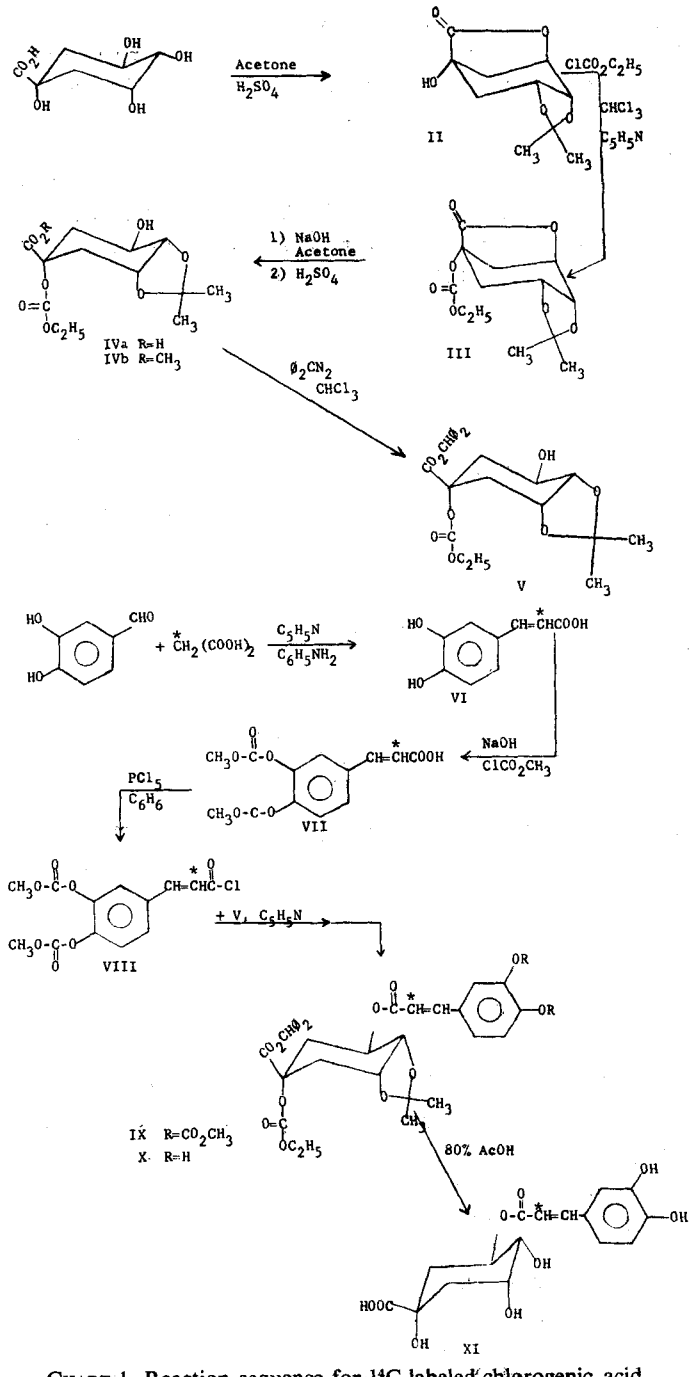

CHART 1. Reaction sequence for ^{14}C-labeled chlorogenic acid.

Caffeic acid-α-^{14}C (VI, 0.467 mCi) was prepared from malonic acid-2-^{14}C (1.96 mCi) [6] and protocatechuic aldehyde by using the method of Johnson [7]. Protection of the phenolic groups giving VII (0.345 mCi) was best achieved by methyl chloroformate. Prior "cold" reaction of VI with phosgene gave a caffeic acid derivative that could not be satisfactorily purified. The acid chloride VIII (0.29 mCi) was easily prepared from VII and reacted with the quinate V to give IX (0.17 mCi). Partial hydrolysis of this material gave X in an impure condition. Further hydrolysis gave chlorogenic acid-α-^{14}C (XI, 30.6 nCi/mg) that was suitable for our studies.

EXPERIMENTAL.

4,5-0-Isopropylidene quinide (II).

The blocked quinide (II), m.p. 139-140° C from acetone, was prepared in 68 % yield from quinic acid (I) by the method of Hanson [5]. The n.m.r. spectrum showed $\delta^{\frac{CDCl}{TMS}}$ 3 : 1.33 (*s*, 3H), 1.50 (*s*, 3H), 1.9-2.8 (*m*, 4H), 3.14 (*s*, 1H), and 4.2-4.9 ppm (*m*, 3H).

Anal. :

$C_{10}H_{14}O_5$ Calcd :	C, 56.07;	H, 6.59
(214.21) Found :	C, 56.39;	H, 6.57

1-0-Ethoxycarbonyl-4,5-0-isopropylidene quinide (III).

The method of Josephson [8] was used to convert II to the carbonate (III), m.p. 107-8° C from ethanol, in 68 % yield. The n.m.r. spectrum showed $\delta^{\frac{CDCl}{TMS}}$ 3 : 1.30 (*t*, 3H), 1.35 (*s*, 3H), 1.52 (*s*, 3H), 2.3-3.2 (*m*, 4H), 4.2 (*q*, 2H), and 4.3-4.9 ppm (*m*, 3H).

Anal.

$C_{13}H_{18}O_7$ Calcd :	C, 54.54;	H, 6.34
(286.29) Found :	C, 54.59;	H, 6.53

1-0-Ethoxycarbonyl-4,5-0-isopropylidene quinic acid (IV).

Several conditions were tried to selectively hydrolyze the lactone moiety of III [3b, 5]. A modified procedure similar to that reported by Haslam *et al.*[9] proved the most successful.

III (30 g, 0.104 mole) dissolved in 100 ml of acetone was added to 105.6 ml of N NaOH. The solution was heated at 50° C for one hour, cooled to 0° C, and 104 ml of N H_2SO_4 slowly added to the cold solution. Most of the acetone was removed at room temperature under reduced pressure and the residue extracted with 4 × 100 ml of ethyl acetate. The organic layer was separated, dried over $MgSO_4$, and stripped to dryness under reduced pressure.

The semi-solid residue was recrystallized twice from ethyl acetate to give 24 g (75 %) of IV*a*, m.p. 155-157° C. The infrared spectrum of IV*a* showed, among others, absorptions at 2.87 μ (hydroxyl group) and 5.80 μ (carboxylic acid). The n.m.r. spectrum in dimethyl sulfoxide-d_6 showed absorptions at 1.18 (*t*, 3H), 1.23 (*s*, 3H), 1.35 (*s*, 3H), 1.6-2.1 (*m*, 4H), 3.7-4.5 (*m*, 3H), and 4.10 ppm (*q*, 2H). The two labile protons of IV*a* were deuterated by the solvent as expected and the amount of DMSO-d_5 in the n.m.r. sample increased the equivalent of two protons.

Anal.

$C_{13}H_{20}O_8$ Calcd :	C, 51.31;	H, 6.67
(304.30) Found :	C, 51.43;	H, 6.54

Diphenylmethyl-1-0-ethoxycarbonyl-4,5-0-isopropylidenequinate (V).

The method of Haslam *et al.* [10] was used to prepare V. A freshly prepared solution of diphenyl diazomethane (16.9 g, 0.82 mole) in 70 ml of chloroform was added to 23 g (0.075 mole) of IV*a* in 350 ml of chloroform. The solution was refluxed for six hours and evaporated to dryness under reduced pressure. The residue was recrystallized twice from ethyl acetate to give 35.6 g (72 % yield) of V, m.p. 164-165° C. The n.m.r. spectrum showed δ^{CDCl}_{TMS} 3 : 1.19 (*t*, 3H), 1.32 (*s*, 3H), 1.49 (*s*, 3H), 1.9-2.8 (*m*, 5H), 4.0 (*q*, 2H), 3.8-4.5 (*m*, 3H), 6.90 (*s*, 1H), and 7.31 ppm (*s*, 10H).

Anal.

$C_{26}H_{30}O_8$ Calcd :	C, 66.36;	H, 6.43
(470.53) Found :	C, 66.33;	H, 6.22

Caffeic acid-α-^{14}C (VI).

The procedure reported by Johnson [7] was the most successful in prior "cold" reactions and was used to prepare specifically labeled VI.

Protocatechuic aldehyde (13.8 g, 0.1 mole), malonic acid-2-^{14}C (23 g, 1.96 mCi), 50 ml of pyridine, and 1.4 g of aniline were mixed and left at room temperature overnight. The resulting black solution was heated at 50-55° C for five hours, cooled to room temperature, and poured in 60 ml of concentrated hydrochloric acid containing 100 g of crushed ice. After warming to room temperature the precipitate was removed and washed with 2 × 20 ml of 5 % HCl followed by 2 × 20 ml cold water. Recrystallization from water yielded 7.7 g (0.467 mCi) of caffeic acid-α-^{14}C (VI), m.p. 223-5° C. In a cold run the IR and n.m.r. spectra of this material was identical with an authentic sample.

Anal.

$C_9H_8O_4$ Calcd :	C, 60.00;	H, 4.48
(180.15) Found :	C, 60.15;	H, 4.44

0,0-dimethoxycarbonyl caffeic acid-α-^{14}C (VII).

Specifically labeled caffeic acid (VI, 7.7 g, 0.467 mCi) was dissolved in 93 ml of N NaOH, cooled to 0-5° C, and 12 ml of methyl chloroformate added dropwise with stirring. After stirring for an additional half hour the precipitate was removed by filtration and mixed with 300 ml of benzene. Slow distillation at atmospheric pressure removed the last traces of water. Gradual cooling deposited 10.34 g of VII (82 %, 0.345 mCi, m.p. 142-46° C) from the benzene solution. This material was used in the following step to prepare the radioactive acid chloride VIII.

0-0-dimethoxycarbonyl caffeoyl chloride-α-^{14}C (VIII).

VII (10.34 g 0.045 mole, 0.345 mCi) was added portionwise to 280 ml of anhydrous benzene containing 10 g of PCl_5. After refluxing for two hours the reaction mixture was evaporated to dryness under reduced pressure. The residue was recrystallized from carbon tetrachloride to give 8.4 g of VIII (m.p. 102-104° C, 77 %, 0.29 mCi). Lit. m.p. 108-9° C. The acid chloride VIII and the protected quinic acid derivative V were reacted together to give IX, the chlorogenic acid precursor.

Diphenylmethyl-1-0-ethoxycarbonyl-3-(caffeoyl-α-^{14}C)-4,5-0-isopropylidenequinate (X).

V (12.6 g) dissolved in 130 ml of anhydrous benzene which contained 13 ml of pyridine was warmed to give a clear solution. To this solution VIII (8.4 g, 0.29 mCi) was added in small portions Pyridine · HCl was deposited immediately from this solution. The mixture was refluxed for 6 hours, cooled to room temperature, washed successively with 3 × 40 ml of 2N HCl, 2 × 40 ml saturated $NaHCO_3$, and 40 ml of water. The organic layer was dried and evaporated to dryness to give 14.2 g of IX (0.17 mCi). This material was dissolved in 200 ml of methanol and 36 ml of N NaOH added dropwise. The resulting dark red mixture was stirred at 30° C for one-half hour, cooled and 150 ml cold water followed by 36 ml of N H_2SO_4 was added. This solution was extracted with ethyl acetate (300 ml 2 × 150 ml), dried, and evaporated to dryness under reduced pressure to give a gummy residue. Recrystallization from benzene gave 2.4 g of X as a yellow powder, m.p. 120-130° C. This compound could not be purified further. In a cold run the analysis was fair.

Anal.

$C_{35}H_{36}O_{11}$ Calcd :	C, 67.31;	H, 6.12
(632.68) Found :	C, 66.17;	H, 6.20

Chlorogenic acid-α-^{14}C.

X (2.4 g) was dissolved in 24 ml of 80 % acetic acid and refluxed for six hours. The solution was evaporated to dryness and the residue dissolved in 20 ml of saturated aqueous sodium bicarbonate. The resulting solution was extracted with ethyl acetate (3 × 25 ml) and the organic layer discarded. The basic solution was made slightly acidic with 2N HCl and again extracted with (3 × 25 ml) ethyl acetate. The aqueous layer was evaporated to dryness and the residue treated with 50 ml of boiling methanol three times. The methanol solution was evaporated to dryness and the residue dissolved in a minimal amount of 2N HCl. This solution was extracted three times with 200 ml portions of ethyl acetate. The organic layer was dried over $MgSO_4$ and evaporated to dryness under reduced pressure. The residue was recrystallized from water to give 0.165 g of crystalline chlorogenic acid-α-^{14}C (XI), m.p. 207-8° C, specific activity 30.6 nCi/mg. In a "cold" run the material at this final stage had identical NMR, I.R., U.V., and R_f values in paper chromatography when compared with an authentic sample of chlorogenic acid.

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