PREPARATION OF QUERCETIN-4-^{14}C

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Summary

The preparation of radiolabelled quercetin proceeds through initial preparation of an alkoxyacetonitrile followed by condensation with phloroglucinol to give the acetophenone, carbonyl-^{14}C. Esterification with veratroyl chloride and rearrangement with triethylamine in refluxing pyridine yields a flavone labelled in position-4. Saponification and dealkylation give quercetin-4-^{14}C.

Keywords: Methoxyacetonitrile-1-^{14}C, benzyloxyacetonitrile-1-^{14}C, quercetin-4-^{14}C, flavonoid synthesis.

INTRODUCTION

The flavonol, quercetin (6) is the most widely occurring flavonoid and is present in varying amounts either as aglycone or in glycosylated form in the diets of animals that consume these plant materials. Nutritional implications of this dietary consumption are not well understood although flavonols such as quercetin are commonly regarded as harmless, and quercetin as its 3-O-rutinoside (rutin) has even received some attention for use in treatment of capillary fragility in humans [2]. More recently, however, the observation that quercetin is mutagenic in the Ames bioassay [3] has raised the question whether ingestion of flavonoids might be, in fact, a source of human cancer and has led to many studies which have at present not yet confirmed this hypothesis[4]. The presence of flavonoid materials in plants is probably associated with defense mechanisms against pathogenic organisms and herbivorous animals (2), and we have observed that quercetin and its glycosides

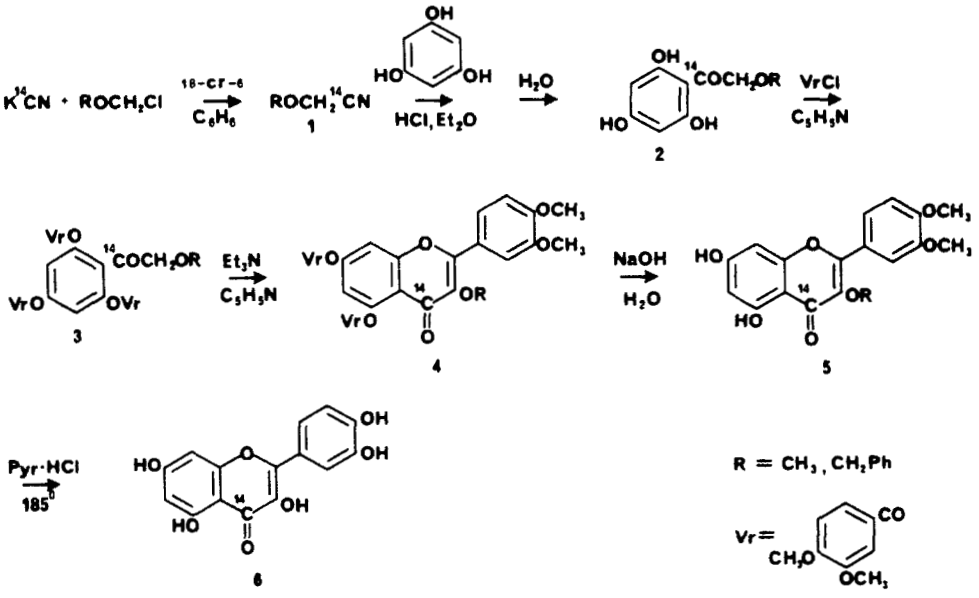
0362-4803/83/040515-07$01.00

Received October 12, 1982
Revised November 24, 1982

are significant larval growth inhibitors in several insect species (5). Studies on the physiological mode of action upon insect larvae and upon the metabolic fate of the flavonoid required radio-labelled quercetin. Since all hydrogens of the quercetin nucleus are at least potentially exchangable (6) it was considered desirable to incorporate ^{14}C into the flavone skeleton. The procedure described in this paper provides this result and constitutes a general method for flavonol-4-^{14}C preparation.

RESULTS AND DISCUSSION

The key intermediate of the synthetic scheme shown is the alkoxyacetonitrile (1) which is prepared in the initial step. Both benzyloxy - and methoxyacetonitrile have usually been prepared by the reaction of a suitable alkylating agent with formaldehyde cyanohydrin (7,8); however, these procedures involve aqueous extractions and are not efficient for small scale



Synthetic Scheme

preparations. Benzyloxyacetonitrile when prepared in this way is also not very pure [7]. The method of choice for preparation of these intermediates relies upon phase-transfer of potassium cyanide mediated by the appropriate crown ether (18-crown-6). It gives excellent yields of benzyloxyacetonitrile from benzyl chloromethyl ether in dichloromethane, benzene or acetonitrile as solvent. Benzene was selected as the most desirable solvent since its low polarity permitted a simple chromatography of the product on silica gel without the need for preliminary evaporation. On a 10 millimole scale using unlabelled material the yield of benzyloxyacetonitrile was 87% after distillation. Preparations of methoxyacetonitrile were carried out in CH_2Cl_2 and in benzene, but the yields were quite variable. In general, the larger the preparation, the better the yield although several one millimole runs gave almost quantitative yields. The lack of reproducibility of the small scale runs made methoxyacetonitrile less desirable as a labelled intermediate; also, its relative volatility compared to benzyloxyacetonitrile led to the choice of the latter compound in the radiochemical sequence.

Condensation of a nitrile with phloroglucinol under Hoesch Reaction conditions generally involves exposure of the reactants and products to anhydrous HCl and $ZnCl_2$ in ether [9]. Since cleavage of benzyl protective groups can occur under similar conditions [10] it was important to reduce contact time to a minimum. Also, the $ZnCl_2$ was omitted to lessen the severity of conditions. A reaction time of 5 hr at room temperature was optimal, and on a 8 mmole scale provided a 57% yield of nonlabelled material. Chromatography of the reaction mixture on Sephadex LH-20 was an effective method of purification giving 2 (R=-CH_2Ph) with only a trace of phloroglucinol remaining.

Esterification of 2 with veratroyl chloride in pyridine was straightforward, and subsequent rearrangement to the flavone system could be carried out after aqueous workup or more conveniently in situ with triethylamine at reflux. After base hydrolysis of the flavone ester 4 a 55% yield of 5 (R=-CH_2Ph) was obtained starting with one mmole of nonradioactive 2. Simultaneous debenzylation and demethylation were effected in pyridine hydrochloride at ca. 185°C to provide quercetin (6) in 75% yield.

In the radioactive sequence starting with one mmole of labelled cyanide, the yields per step were lower and resulted in an overall conversion of 9% based on $K^{14}CN$.

EXPERIMENTAL

Radioactivity measurements were carried out using a Packard Tri-Carb 460C system and Aquasol-2 universal LSC cocktail (NEN). Nuclear magnetic resonance spectra were measured at 90MHz on a Varian EM-390 spectrometer using $CDCl_3$-DMSO-d_6 solvent and TMS as internal standard. Nonradioactive samples were used from preliminary trials. $K^{14}CN$ (41.5mCi/mmole) was purchased from ICN Radiochemicals Div. and was admixed with nonradioactive material to a specific activity of ca. 2 mCi/mmole. Thin layer chromatography employed silica gel-60, F-254 (E. Merck) using 97% formic acid, ethyl acetate, benzene (5:10:40).

2-Benzyloxy-2',4',6'-trihydroxyacetophenone-1-^{14}C (2, R=-CH_2Ph)--To a suspension of $K^{14}CN$, 65.0 mg (2.00 mCi, 1 mmole), in 0.5 ml of anhydrous benzene (from 4Å molecular sieve) were added 10 mg of 18-crown-6 ether and 170 mg (1.09 mmole) of benzyl chloromethyl ether (11). The mixture was stirred magnetically 1 hr at ambient temperature, and an additional 0.5 ml of benzene was used to wash down solid from the walls of the reaction vessel. After 16 hr

stirring time, the entire reaction mixture was transferred to a column of silica gel 8 mm dia x 20 mm in a disposable tube and benzyloxyacetonitrile (1, R=-CH_2Ph) was eluted with 7 ml of benzene. The colorless oil obtained by evaporation of solvent on the rotary evaporator was taken up in 5 ml of ether and 152 mg (1.2 mmoles) of phloroglucinol was added. HCl gas was passed in for 3 min, and the resulting solution was stirred for 5 hr during which time a solid crust formed on the flask walls. The supernatant ether was pipetted off, and the solid was washed with 5 ml of ether. Water, 5 ml, was added to dissolve the remaining solid and this mixture was stirred at 90-95° for 30 min to give an oily layer. Most water was removed under reduced pressure and the remaining orange oil was applied to a 50 mm dia x 950 mm Sephadex LH-20 column in methanol. The desired ketone (2, R=-CH_2Ph) appeared in elution vol. 2375-2675 ml (0.91 mCi, 45%). TLC showed one major component of R_f=0.22 with minor impurity of R_f=0.12 corresponding to phloroglucinol. PMR of 2: δ (ppm) 7.2-7.5 (5H, complex, benzyl aromatic) 5.88 (2H, s, aromatic), 4.75 (2H,s,-CH_2-) and 4.64 (2H, s,-CH_2-). M.p. 131-134°C (toluene-EtOAc).

3-Benzyloxy-5,7-dihydroxy-3',4'-dimethoxyflavone-4-^{14}C (5, R=-CH_2Ph)--In a 50 ml flask equipped with stirrer, condenser and drying tube were placed 0.81 mCi (0.4 mmole) of 2 and 5 ml of anhydrous pyridine (from 4A molecular sieve). Veratroyl chloride, 402 mg (2.0 mmoles), was added, and the mixture was refluxed 30 min. The flask was cooled briefly, and 5 ml of triethylamine was added. After 16 hr reflux the reaction mixture was then evaporated to a paste on the rotary evaporator. A solution of 0.25 g of NaOH in 10 ml of 50/50 MeOH-H_2O was added, and the suspension of solids was warmed to reflux 30 min to give a homogeneous solution which was then taken to dryness under reduced pressure. The resulting mass was dissolved in 10 ml of H_2O and CO_2

was passed in carefully to avoid frothing until precipitation of solid was complete. After collection by filtration, the sticky brown product was dissolved in 20 ml of ethyl acetate and the solution was evaporated in vacuo to remove residual water. Addition of 25 ml ethyl acetate now gave a solution which contained dark suspended material, and filtration yielded a clear yellow solution of 5, 0.34 mCi (42%) showing one major component of R_f=0.47 upon TLC with minor impurities of R_f's corresponding to phloroglucinol and to 2. Crystallization of nonradioactive 5 from EtOAc-hexane gave material of mp 202-204°C. PMR: δ (ppm) 7.64 (1 H, d,d, $J_{5',6'}$=9, $J_{2',6'}$=2 Hz, H6'), 7.15-7.45 (6 H, complex, H-2' and pendant phenyl) 6.90 (1 H, d, $J_{5',6'}$=9 Hz, H-5') 6.39 (1 H, d, $J_{6,8}$=2 Hz, H-6 or 8), 6.28 (1 H, d, $J_{6,8}$=2 Hz, H-6 or 8), 5.03 (2 H, s, 3-O-CH_2-), 3.90 (3 H, s,-OCH_3), 3.67 (3 H, s,-OCH_3).

Quercetin-4-^{14}C (6)--A solution of 5 (0.32 mCi, 0.16 mmole) in EtOAc was taken to dryness in a 10 ml flask. Pyridine hydrochloride, 1 g, was added and the stirred mixture was warmed for 2 hr in an oil bath maintained at 180-190°C. Sublimation of pyridine hydrochloride onto the upper surfaces of the flask occurred during this step, and the flask was lowered in the oil bath at 0.5 hr intervals to melt and return the sublimate to the pot. The flask was cooled to 50°C and 8 ml of MeOH was added. The resulting solution was applied to a 50 mm dia x 950 mm Sephadex LH-20 column and elution was carried out with MeOH. Quercetin (6) emerged in elution vol 4750-5250 ml (0.144 mCi, 46%) and was homogeneous by TLC, R_f=0.19, co-chromatography with authentic material. Determination of concentration of 6 in solution by measurement of A_{370nm} showed that its specific activity was 2.07 mCi/mmole.

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