SYNTHESES OF ^{14}C-LABELED CINNAMIC, MANDELIC, PHENYLACETIC, PHENYLGLYOXYLIC, AND PHENYLPYRUVIC ACIDS.

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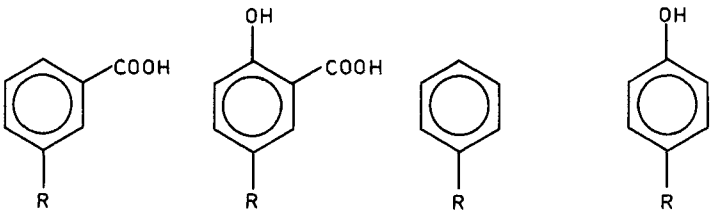
SUMMARY

Syntheses are described for 3'-carboxyphenylpyruvic acid-2-C14, 3'-carboxy-4'-hydroxyphenylpyruvic acid-2-C14, (E)-3'-carboxycinnamic acid-2-C14, (E)-3'-carboxy-4'-hydroxycinnamic acid-2-C14, 3'-carboxyphenylacetic acid-1-C14, 3'-carboxy-4'-hydroxyphenylacetic acid-1-C14, 3'-carboxymandelic acid-1-C14, 3'-carboxy-4'-hydroxymandelic acid-1-C14, 3'-carboxyphenylglyoxylic acid-1-C14, and 3'-carboxy-4'-hydroxyphenylglyoxylic acid-1-C14.

INTRODUCTION

3-(3-Carboxyphenyl)alanine (Ia), 3-(3-carboxy-4-hydroxyphenyl)alanine (Ib), (3-carboxyphenyl)glycine (IIa), and (3-carboxy-4-hydroxyphenyl)glycine (IIb) occur free in various plants.[1,2] In continuation of studies on the biosynthesis of these amino acids[1,3] the need arose for labeled material of the corresponding keto acids and of possible intermediates in the previously established[1] transformation of the alanine side chain of Ia-b into the glycine side chain of IIa-b. The present paper describes the preparation of these compounds, i. e. ^{14}C-labeled 3'-carboxyphenylpyruvic acid (IIIa), 3'-carboxy-4'-hydroxyphenylpyruvic acid (IIIb), (E)-3'-carboxycinnamic acid (IVa), (E)-3'-carboxy-4'-hydroxycinnamic acid (IVb), 3'-



a b c d

I: R = $CH_2\overset{+}{C}H\overset{+}{N}H_3COO^-$

II: R = $CH\overset{+}{N}H_3COO^-$

III: R = $CH_2COCOOH$

IV: R = (E)-CH=CHCOOH

V: R = CH_2COOH

VI: R = CHOHCOOH

VII: R = COCOOH

carboxyphenylacetic acid (Va), 3'-carboxy-4'-hydroxyphenylacetic acid (Vb), 3'-carboxymandelic acid (VIa), 3'-carboxy-4'-hydroxymandelic acid (VIb), 3'-carboxyphenylglyoxylic acid (VIIa), and 3'-carboxy-4'-hydroxyphenylglyoxylic acid (VIIb).

DISCUSSION

Labeled phenylpyruvic acid (IIIc) has previously been prepared from benzaldehyde-CHO-C^{14} and acetylglycine[4] and by enzymatic oxidation of labeled phenylalanine.[5] Labeled p-hydroxyphenylpyruvic acid (IIId) has previously been prepared from p-hydroxybenzaldehyde and acetylglycine with labeling either from the aldehyde group[6] or from the glycine carboxyl group.[7] and by condensation of p-hydroxybenzaldehyde and hydantoin, followed by hydrolysis in base.[8] Because of lack of easy access to the appropriate ^{14}C-labeled aldehydes, IIIa and IIIb were produced from the unlabeled aldehydes and acetylglycine-2-C^{14} by the classical Erlenmeyer synthesis. This method avoids the use of base, important on a micro scale since IIId and other phenylpyruvic acids are easily autoxidised above pH 7 to give benzaldehydes.[8,9] The macro-scale syntheses and properties of IIIa and b have previously been described.[10]

Labeled cinnamic acid (IVc) and p-coumaric acid (IVd)

have previously been prepared by Knoevenagel condensations.[11, 12] Labeled IVc has also been prepared from phenylalanine by action of phenylalanine ammonia-lyase.[13] IVa and IVb have previously been prepared by Knoevenagel condensations.[14,15] The labeled compounds were easily produced from isophthalaldehydic acid or 5-formylsalicylic acid and malonic acid-2-C14. This condensation invariably produces the trans (E) form.[16]

Labeled phenylacetic acid (Vc) has previously been prepared by the Grignard reaction with $^{14}CO_2$[17] and from benzoyl chloride and diazomethane.[18] Labeled p-hydroxyphenylacetic acid (Vd) has been obtained from p-methoxybenzylchloride and cyanide with subsequent hydrolysis and demethylation[19] and by enzymatic oxidation of tyrosine.[20] Va has previously been prepared from α-bromo-m-tolunitrile and cyanide with subsequent hydrolysis.[21] Va-1-C14 was easily prepared in the same way with sodium cyanide-C14. Vb has previously been synthesized from 5-formylsalicylic acid and cyanide with subsequent reduction with HI/P and hydrolysis,[22] and from 3'-carboxy-4'-hydroxyphenylacetonitrile by hydrolysis.[23] In the present study it was found most advantageous to produce Vb-1-C14 by reduction of VIb-1-C14 (see below) with HI.

Labeled mandelic acid (VIc) has previously been synthesized from benzaldehyde by the cyanohydrin method[11] and by internal disproportionation of phenylglyoxal.[24] The synthesis of labeled p-hydroxymandelic acid (VId) seems not to have been reported. VIa and VIb have not been described previously. The cyanohydrin corresponding to VIa has been prepared from isophthalaldehydic acid and cyanide.[22] VIa-1-C14 and VIb-1-C14 were produced by cyanohydrin syntheses from the appropriate aldehydes and sodium cyanide-C14.

Labeled phenylglyoxylic acid (VIIc) has been produced from benzoyl chloride and cuprous cyanide via the nitrile.[25] Labeled p-hydroxyphenylglyoxylic acid (VIId) has not been described. Whereas the synthesis of VIIa has not been reported, the synthesis of VIIb by permanganate oxidation of 5-acetylsalicylic acid has been described.[26] Both compounds exist as stable hydrates, possibly diols. VIIa-1-C14 and VIIb-1-C14

were both produced by permanganate oxidation of the corresponding mandelic acids (see above).

The purity of all materials was monitored by paper chromatography followed by autoradiography. Furthermore all syntheses were first performed with unlabeled material and the identity of the products established by m.p., IR-spectra and chromatographic behaviour. Macrosyntheses followed by elementary analyses were performed for VIa, VIb, and VIIa, previously undescribed compounds.

CONCLUSION

The syntheses of a number of ^{14}C-labeled aromatic carboxylic acids have been performed using easily available ^{14}C-labeled starting materials (glycine, cyanide, malonic acid). Some of the syntheses described are variations of methods previously used for the production of labeled compounds, whereas the syntheses of Vb, VIIa and VIIb are new. Success in the isolation of pure crystalline products depends in most cases on the addition of appropriate amounts of unlabeled material to the reaction mixture before the final purification. This causes lower specific activity in the products but certainly is preferable to large-scale syntheses involving more radioactivity.

EXPERIMENTAL

Paper chromatography was performed by the descending technique on Whatman No. 1 paper in the following solvents: A: EtOH-H_2O-conc. NH_3 (16:3:1), B: $CHCl_3$-MeOH-HCOOH-H_2O (16:2:1:1), and C: n-BuOH-AcOH-H_2O (12:3:5). The compounds were identified on the chromatograms by inspection under UV-light. Autoradiography was performed by exposing the chromatograms to Kodak Kodirex X-ray films.

^{14}C-KCN, sodium malonate-2-C14, and glycine-2-C14 were obtained from Amersham, England. Specific activities were determined by weighing small amounts of materials on a microbalance and liquid scintillation counting on a Packard 3320 instrument. Counting efficiency was determined by use of an external standard.

UV-spectra were determined on unlabeled materials for all compounds on a Zeiss DMR-21 instrument at 17° in 0.1 N HCl. Previously unreported data are listed under the syntheses of the labeled compounds.

PMR-spectra of IVa and IVb were determined on a JEOL C-60HL instrument in deutero-DMSO with TMS as reference. The olefinic protons occurred as doublets (J = 15.8 Hz for both IVa and IVb) at δ 6.4 (C_2-proton, IVa), 6.3 (C_2-proton, IVb), 7.5 (C_3-proton, IVa), and 7.5 (C_3-proton, IVb). These values support the (E) (trans) configurations.[27]

IIIa-2-C14 (in the enol form). Glycine-2-C14 (3.9 mg, 250 μCi) was acetylated as described in the literature to give acetylglycine-2-C14 (5.1 mg, 84%). Acetylglycine-2-C14 (2.1 mg), isophthalaldehydic acid methyl ester (m.p. 51°, 3.2 mg), potassium acetate (2 mg), and acetic anhydride (15 μl) were heated in a closed microtube to 90-100° for 2.5 hrs, cooled and placed in the refrigerator overnight. After addition of water (100 μl) the crystalline product was isolated by centrifugation, washing with water and drying to give yellow crystals (3.8 mg) of 4-(3-carbomethoxybenzylidene)-2-methyl-2-oxazolin-5-one. The crude azlactone was refluxed under nitrogen with 50% ethanol (400 μl) and 8 N HCl (100 μl) for 7 hrs. Unlabeled IIIa (9 mg) and water (2 ml) were added and after decolourization with charcoal the solution was left in the refrigerator. After 3 days a small precipitate was removed by filtration and the solution concentrated in vacuo to 0.5 ml. IIIa crystallized at 0° and was isolated by filtration. Yield 5.3 mg, specific activity 4.3 μCi/mg (radiochemical yield from acetylglycine 27%). R_f in solvent B: 0.36.

IIIb-2-C14 (in the enol form). 4-(3-Carbomethoxy-4-acetoxybenzylidene)-2-methyl-2-oxazolin-5-one was prepared as described above from acetylglycine-2-C14 (2.0 mg, 78 μCi), 5-formylsalicylic acid methyl ester sodium salt (3.7 mg), potassium acetate (1.6 mg), and acetic anhydride (18 μl) by heating to 97-110° for 2 hrs. Yield of yellow crystals 3.9 mg (85%). The azlactone was refluxed under nitrogen with 25% ethanol (800 μl)

and 10% HCl (100 μl) for 14 hrs. Unlabeled IIIb (10 mg) and water (2 ml) were added. After a night at 0° a small precipitate was removed by centrifugation. After several days in the refrigerator IIIb was isolated by centrifugation. Yield 5.9 mg, specific activity 4.8 μCi/mg (radiochemical yield from acetylglycine 35%). R_f in solvent B: 0.35.

IVa-2-C14. To sodium malonate-2-C14 (15 mg, 163 μCi) in water (1 ml) was added conc. HCl (25 μl). The solution was evaporated to dryness under reduced pressure. Isophthalaldehydic acid (15 mg), pyridine (200 μl), and piperidine (10 μl) were added. The reaction mixture was heated under nitrogen to 80-90° for 30 min and to 120-30° for 2 hrs, cooled, and dissolved in water (200 μl). After acidification with 5% HCl (1 ml), IVa precipitated. Final purification was accomplished by recrystallization from MeOH-H_2O (1:2). Yield 12.2 mg, specific activity 7.9 μCi/mg (radiochemical yield 59%). R_f in solvent B: 0.63. λ_{max} 272 nm, ε_{max} 20.000, and 230 nm, ε_{max} 21.000.

IVb-2-C14. Malonic acid-2-C14, obtained as described above from sodium malonate-2-C14 (15 mg, 163 μCi), was mixed with 5-formylsalicylic acid (17 mg) and 75% sulfuric acid (150 μl) and heated under nitrogen to 80-90° for 1 hr and to 90-100° for 1 hr. After cooling, water (1.5 ml) was added and the crystalline product isolated by centrifugation and washing twice with water. After two recrystallizations from acetone-water (1:3) pure material was obtained. Yield 8.2 mg, specific activity 6.1 μCi/mg (radiochemical yield 31%). R_f in solvent A: 0.43; in solvent B: 0.62. λ_{max} 293 nm, ε_{max} 22.000, and 233 nm, ε_{max} 20.000.

Va-1-C14. Sodium cyanide-C14 (5.4 mg, 150 μCi) in water (100 μl) was mixed with α-bromo-m-tolunitrile (19.6 mg) in ethanol (300 μl). After reflux for 2 hrs the solution was evaporated to dryness, the residue dissolved in ether (500 μl) and conc. HCl (500 μl) added, and the solution left for 2 days at room temperature and finally heated on a boiling water bath for 2 hrs. The solution was extracted with ethyl acetate and the

ethyl acetate washed with water, dried, and evaporated. After addition of 10 mg of unlabeled Va, two recrystallizations from 20% HCl produced a pure sample. Yield 15.1 mg, specific activity 4.1 μCi/mg (radiochemical yield 41%). R_f in solvent B: 0.57. λ_{max} 278 nm, ε_{max} 1200, 232 nm, ε_{max} 11.000, and 201 nm, ε_{max} 31.000.

Vb-1-C14. A mixture of VIb-1-C14 (8.5 mg, 51 μCi), HI (d 1.7, 300 μl) and red P (100 mg) was refluxed for 4 hrs. 4 N HCl (2 ml) was added twice with subsequent evaporation under reduced pressure. Ether (10 ml) was added to the residue, and after filtration, washing with water, and drying, the solution was evaporated to dryness. Unlabeled Vb (4.4 mg) was added and the material recrystallized three times from water. Yield 10 mg, specific activity 4.3 μCi/mg (radiochemical yield 84%). R_f in solvent B: 0.66. λ_{max} 307 nm, ε_{max} 3300, and 207 nm, ε_{max} 35.000; shoulder at 237 nm.

VIa. The bisulfite adduct of m-cyanobenzaldehyde (3 g) was added with stirring to a solution of sodium cyanide (0.48 g) in water (30 ml) during 15 min at 0°. The precipitated m-cyanomandelonitrile was collected by filtration, washed with cold water and mixed with conc. HCl (25 ml). After stirring for 2 hrs at room temperature, the mixture was left for 48 hrs and heated on a boiling water bath for 4 hrs. After several hours at 0° the colourless crystals of VIa were collected by filtration. Yield 0.93 g (47.5%), m.p. 195-6°. Recrystallization from water produced an analytical sample, m.p. 205-6°. Found: C 55.24; H 4.24. Calc. for $C_9H_8O_5$: C 55.10; H 4.08%. λ_{max} 276 nm, ε_{max} 900, 229 nm, ε_{max} 10.800, and 202 nm, ε_{max} 33.000.

VIa-1-C14. Sodium cyanide-C14 (4.9 mg, 1035 μCi) in water (0.5 ml) was mixed with the bisulfite adduct of m-cyanobenzaldehyde (35 mg) and the mixture stirred for 30 min at 0°. 30% $NaHSO_3$ (100 μl) was added and the cyanohydrin extracted with ether (10 ml). The ether extract was washed with water, dried, and evaporated to dryness. Ether (1 ml) and conc. HCl (1 ml) were added. After stirring for 3 hrs at room tempera-

ture, and reflux on a boiling water bath for 4 hrs unlabeled VIa (20 mg) was added. The hot solution was filtered. After cooling overnight the crystalline precipitate was isolated by filtration and recrystallized twice from 20% HCl. Yield 18.7 mg, specific activity 14 μCi/mg (radiochemical yield 25%). R_f in solvent A: 0.58, in solvent B: 0.16.

VIb. 3'-Carboxy-4'-hydroxymandelonitrile (0.58 g) in ether (20 ml) was mixed with conc. HCl (20 ml). After stirring for 72 hrs at room temperature and heating for 2 hrs, the solution was filtered and left at 0°. The crystalline precipitate was isolated by filtration. Yield 0.48 g (75%). m.p. 172-4°. Recrystallization from water produced an analytical sample, m.p. 180-1°. Found: C 50.52; H 3.93. Calc. for $C_9H_8O_6$: C 50.94; H 3.77%. λ_{max} 305 nm, ε_{max} 3300, and 208 nm, ε_{max} 35.000, shoulder at 235 nm.

VIb-1-C14. To sodium cyanide-C14 (4.9 mg, 1035 μCi) in water (0.5 ml) was added the bisulfite adduct of 5-formylsalicylic acid (40 mh), and the reaction mixture was stirred for 30 min at 0°. After the addition of conc. HCl (20 μl) the nitrile was extracted with ethyl acetate (15 ml). The extract was washed with water, dried, and evaporated. The crystalline residue was extracted twice with ether (0.5 ml each time). Conc. HCl (1 ml) was added to the ether extract, and after stirring for 3 hrs at room temperature, standing 48 hours at room temperature, and 4 hrs on a boiling water bath unlabeled VIb (10 mg) was added. The hot solution was filtered, and after a few days in the ice box the crystals of VIb were isolated by centrifugation and purified by recrystallization from 4 N HCl. Yield 10.9 mg, specific activity 13.8 μCi/mg (radiochemical yield 15 %). R_f in solvent B: 0.21.

VIIa. To a solution of VIa (m.p. 195-6°, 157 mg) in water (4 ml) and 10 N NaOH (0.4 ml) at -2° was added a solution of potassium permanganate (87 mg) in water (2 ml). After stirring for 2 hrs at -2° and 1 hr at room temperature, 1 ml of ethanol was added. After a few hours the reaction mixture was filtered

and the filtrate adjusted to pH 5 with HCl, concentrated in vacuo to 5 ml, acidified with conc. HCl (1 ml) and extracted with ether (50 ml). The ether extract was washed with water, dried, concentrated to 2 ml, filtered and diluted with hexane. The colourless crystals of VIIa precipitated were collected by filtration. Yield 99 mg (52%), m.p. 148-50° (decomp.). Recrystallization from methyl ethyl ketone-hexane produced an analytical sample, m.p. 159-62° (decomp.). Found: C 51.05; H 3.96. Calc. for $C_9H_6O_5$,H_2O: C 50.94; H 3.77%. λ_{max} 277 nm (shoulder), and 213 nm, ε_{max} 31.000.

VIIa-1-C14. To VIa-1-C14 (6 mg, 84 μCi) in 0.5 M KOH (200 μl) at 0° was added dropwise and with stirring $KMnO_4$ (3.2 mg) in 0.5 M KOH (1 ml) over a period of 15 min. The reaction mixture was stirred for 2 hrs at 0° and for 1 hr at room temperature. Excess permanganate was destroyed by the addition of ethanol (0.1 ml). After filtration and washing with water unlabeled VIIa (10 mg) was added to the filtrate. The solution was adjusted to pH 5 with HCl and concentrated to 1 ml. Conc. HCl (0.1 ml) was added and the product extracted with ether (15 ml). The ether extract was washed with water, dried, and taken to dryness. The solid residue was recrystallized from ether-hexane. Yield 8.6 mg, specific activity 3.5 μCi/mg (radiochemical yield 36%). R_f in solvent C: 0.57.

VIIb-1-C14. VIb-1-C14 (8.3 mg, 115 μCi) was oxidised as described for VIa. Before recrystallization unlabeled VIIb (25 mg) was added. Final purification was accomplished by four recrystallizations from benzene. Yield 7.5 mg, specific activity 0.44 μCi/mg (radiochemical yield 3%). λ_{max} 281 nm, ε_{max} 13.000, and 217 nm, ε_{max} 21.000. R_f in solvent C: 0.48.

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