Chemical Synthesis of α-Formylphenylacetic Acid, the Postulated Precursor of Tropic Acid

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α-Formylphenylacetic acid, the postulated immediate precursor of tropic acid, has been synthesized by deacetalization of α-diethoxymethylphenylacetic acid in the presence of silica gel. The compound was reasonably stable in organic solution. In aqueous media, however, a pronounced lability of this semialdehyde was observed at various pH-values ($t/2 = 4.5$ min at pH 7.4). It is thus very unlikely that this compound can be employed successfully in biosynthetic studies.

Introduction

The biosynthesis of tropic acid (3-hydroxy-2-phenylpropionic acid), the acidic moiety of the tropane alkaloids hyoscyamine and scopolamine, is still largely unknown. From various tracer studies, most authors consider L-phenylalanine to be the general precursor of this acid. Such a conversion would involve three principal reactions: a deamination (or transamination), an isomerization and a reductive step. Although the actual sequence of these events is still obscure, most hypothetical biosynthetic schemes presented to date assume α-formylphenylacetic acid (phenylmalonic semialdehyde) as the immediate precursor of tropic acid [1–6]. The hydroxyl group of the latter compound would thus originate from the reduction of the precursor's aldehyde function by an alcohol dehydrogenase-type reaction.

In contrast to these frequent postulates, no serious attempts to demonstrate the intermediacy of formylphenylacetic acid have been published, a fact which certainly must be attributed to the pronounced lability of formylacetic acids. Kalyanaraman et al. [7] used the stable ethyl α-formylphenylacetate as a substrate, and the negative results obtained by these authors may be explained by the marked difference between the charged free acid and its uncharged ester derivative, the latter not accepted as a substrate by the postulated enzyme.

When we decided to test the presumed role of α-formylphenylacetic acid more closely, it was very surprising that we were unable to find published procedures for the synthesis of this compound. As a prerequisite for further studies on this question we were confronted with the problem of developing a suitable method for the preparation of this eventual precursor of tropic acid.

Experimental

Analytical methods

Thin-layer chromatography was carried out on silica-gel plates with the solvents (I) ethanol: ammonia: water = 78:13:9 and (II) toluene: ethyl formate: formic acid = 5:4:1. Esters were stained by treatment with 2 M hydroxylamine, pH 6, and FeCl3. Aldehydes were detected by spraying with a saturated solution of 2,4-dinitrophenylhydrazine in 1 N HCl, acetalts after pretreatment with 5 N HCl.

GLC-analyses were carried out using 2 m x 2 mm i.d. glass-columns packed with 3% GE-SE 30 on chromosorb WAW-DMCS, 80–100 mesh; gas-flow 40 ml/min; temperature program 80–200 °C (10 °C/min); FID. Samples bearing free carboxyl groups were pretreated by methylation with diazomethane in ether at $-10$ °C.

Mass spectra were recorded on a Varian MAT 711 (70 eV). C,H-analyses were performed by Mikroanalytisches Laboratorium Pascher, Bonn.
Ethyl α-dioethoxymethylphenylacetic acid

Ethyl α-dioethoxymethylphenylacetic acid (6 ml) was hydrolyzed at room temperature for two days in a mixture of 24 ml ethanol and 6 ml 30% aq. KOH [9]. The crude product was recrystallized from benzene/light petrol (prisms; m.p. 135–136 °C; lit. [9] 130–131 °C; yield 60–74%). After thin-layer chromatography, the acid showed a single spot which stained positively with 2,4-dinitrophenylhydrazine. GLC of the methylated derivative indicated purities of 98–100%. Analysis gave 65.39% C; 7.61% H (calcd. for C_{13}H_{18}O_4: 65.53% C; 7.61% H). The structure was further confirmed by mass-spectrometry.

α-Formylphenylacetic acid

The protecting acetal groups of α-dioethoxymethylphenylacetic acid were removed by treatment with wet silica gel [12]. 5 N HCl (0.06 ml) was added with continuous magnetic stirring to a suspension of 600 mg silica gel 60 (70–230 mesh; Merck) in 1 ml CH_{2}Cl_{2}. After 2–3 min, the acetal (50 mg) was added. The mixture was stirred at 30 °C for 30 min, filtered with suction and the solid washed with CH_{2}Cl_{2}. After evaporation of the solvent under a stream of N_{2}, the residue was redissolved in methanol and stored at −20 °C.

For analyses, the semialdehyde preparations were converted to the methyl ester and subjected to GLC; purities of 60–80% were determined under these conditions. GLC-MS analysis of the methyl derivative: m/e 178 (44%, M^+), 150 (26, -CO), 146 (100, -CH_{2}OH), 118 (65, C_6H_{12}O, 91 (80, C_7H_7), 90 (96, C_7H_8), 77 (23). In initial experiments, significant amounts of the methyl ether of the tautomeric enol-form of methyl α-formylphenylacetic acid were observed. GLC-MS: m/e 308 (35%, M^+), 293 (85, -CH_3), 219 (12, -OTMS), 191 (42, CH(OTMS)_2), 147 (100).

Results and Discussion

The reactions by which we synthesized α-formylphenylacetic acid are summarized in Scheme I. Ethyl α-dioethoxymethylphenylacetic acid, the first intermediate in this sequence, was prepared by two different procedures. In the first method, ethyl phenylacetate was formylated to ethyl α-formylphenylacetate [8]; the carbonyl of this compound was subsequently protected by acetalization with ethanol [9] (over-all yields 20–27%). The resulting ester-acetal could also be synthesized directly from ethyl α-bromophenylacetate in 21% yield by the adaptation of a published procedure for the synthesis of methylmalonic semialdehyde [11]. Recoveries and purities of the product obtained by either method were comparable. The second procedure, however, had the advantage of saving one reaction-step. After alkaline hydrolysis of the ester-acetal [9], we were able to isolate very pure crystals of α-dioethoxymethylphenylacetic acid, the stable immediate precursor of the desired semialdehyde.

Serious problems were encountered in the last step of the reaction sequence. A wide variety of conventional deacetalization techniques, using various mineral acids or organic acids, were checked for their applicability in the liberation of α-formylphenylacetic acid. We found that mild reaction conditions (i.e. low H^+ concentrations, low temperatures, short incubation periods) left the acetal more or less unaltered. Under more drastic conditions, on the other hand, the protecting acetal residues were split.
off readily, yielding both the semialdehyde and its tautomeric enol-form, but these reactions were accompanied by an unacceptable degradation of these compounds as indicated by the prevalent formation of phenylacetaldehyde and various other degradation products (e.g. phenylactic acid, mandelic acid, benzoyleformic acid). Finally, the deacetalization method of Huet et al. [12] with wet silica gel came to our attention. This procedure provided an efficient, and yet mild, tool for the synthesis of the free semialdehyde. Using this method, α-diethoxy- methylphenyl acetic acid was deacetalized nearly quantitatively, and only negligible amounts of contaminating degradation products were observed (Fig. 1).

Next, we studied the stability of α-formylphenylacetic acid. In a first set of experiments, a methanolic solution of this compound was kept at different temperatures. Analysis in 1-day-intervals revealed that no significant degradation had occurred after 4 days at −21 °C, +4 °C, or at room-temperature. Even at 30 °C, only ca. 40% of the semialdehyde decarboxylated to phenylacetaldehyde within this period; the data corresponded to a half-life ($t/2$) of about 5 days. In subsequent experiments, we analyzed the stability of α-formylphenylacetic acid in aqueous solution (50% methanol) at various pH-values at 30 °C. Under these conditions, the lability of this compound was drastically enhanced. At pH 1.9, the initial semialdehyde concentration dropped to 73% within 1 h, to 59% after 2 h, and to 41% after 3 h ($t/2 = 2.3$ h). At pH 4.0, we observed losses of 50% in 20 min; and at pH 7.4, 40% and 90% were degraded after 3 min and 15 min, respectively ($t/2 = 4.5$ min). In more alkaline media, the compound was destroyed completely within a few minutes.

Summarizing these results, it is evident that α-formylphenylacetic acid can be synthesized in satisfactory yield and purity. In contrast to its acceptable stability in organic solution, this semialdehyde exhibits an extreme lability in aqueous media, particularly at physiological H⁺-concentrations. It thus appears impossible to use this compound in feeding experiments or to detect it as the product of an
enzymatic reaction. It is also very doubtful whether this compound can be employed successfully as an enzyme substrate. Preliminary experiments in our laboratory on the reduction of the semialdehyde to tropic acid with enzyme preparations from Datura stramonium or Atropa belladonna indicated only the formation of phenylethanol. It must be assumed that this alcohol was formed by the reduction of phenylacetaldehyde, the main degradation product of α-formylphenylacetic acid.

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