

PREPARATION OF RADIOACTIVELY LABELLED INDOLE-3-ACETIC ACID PRECURSORS.

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Abstract:

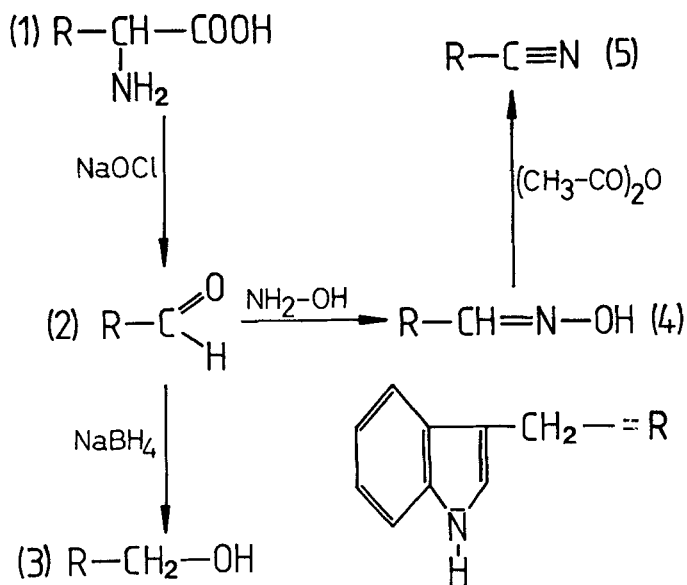
A microscale method for the synthesis of indole-3-acetaldehyde, indole-3-ethanol, indole-3-acetaldoxime and indole-3-acetonitrile from radioactively labelled tryptophan with high yield and no reduction in specific radioactivity is described.

Introduction:

Indole-3-acetaldehyde, indole-3-ethanol, indole-3-acetaldoxime, and indole-3-acetonitrile are important intermediates in indole-3-acetic acid (auxin) biosynthesis of plants. All these compounds have been prepared chemically on large scale versions (1,2,3,4). However, no convenient procedures exist for microscale preparation with reasonably high yield and high purity (>90%) without extensive purification involving unwanted losses during the preparation. Especially the preparation of small amounts of labelled compounds from expensive precursors presupposes such procedures.

We describe microscale methods to synthesize the indole derivatives at high specific radioactivity exclusively from tryptophan which is available in different radiolabelled versions.

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tryptophan (1), indole-3-acetaldehyde (2), indole-3-ethanol (3),
indole-3-acetaldoxime (4), indole-3-acetonitrile (5)

I. Preparation of Indole-3-acetaldehyde.

The reaction is carried out in a cylindrical vessel (100x25 mm) mounted in a waterbath at 45°C. The reaction medium is prepared by saturating 1 M sodium carbonate solution successively with sodium chloride and mannitol at 25°C. The pH is adjusted to 10.0 with 2 M sodium hydroxide. 5-10 μmol of tryptophan at the specific radioactivity desired for the product are dissolved in the reaction vessel in 500 μl of the described reaction medium. 10 ml of freshly distilled benzene (p.a. grade) are added and the 2 phases thoroughly mixed with a magnetic stirrer. 240 μl of 0.5% sodium hypochlorite solution, which has been saturated with sodium chloride, are added at a rate of 4 $\mu\text{l}/\text{min}$. Every 10 minutes the benzene phase is pipetted off and replaced by fresh benzene. The combined benzene phases are dried on sodium sulphate and reduced by rotary film evaporation to the desired volume. The preparation is stored at 4°C.

II. Preparation of Indole-3-ethanol.

10 ml of the dried benzene phase of preparation I are added to 1 ml 1 M sodium borohydride in the same type of reaction vessel and the two layers are stirred for 10 min at 25°C. The benzene phase is pipetted off and processed for storage as described for preparation I.

III. Preparation of Indole-3-acetaldoxime.

The dried benzene phase of preparation I is added to 1 ml 100 mM hydroxylamine, pH 7.0, in the same type of reaction vessel and the two layers are stirred for 1 hour at 25°C. The benzene phase is pipetted off and processed for storage as described for preparation I.

IV. Preparation of Indole-3-acetonitrile.

To 10 ml of the dried benzene phase from preparation III 10 mmol acetanhydride are added and the reaction mixture is stirred at 25°C for 10 min. To eliminate unreacted acetanhydride and acetic acid formed during the reaction from the benzene phase 1 M NaOH is added

Educt	Product	Yield % [×]	Radiochemical Purity of Product %
R-CHNH ₂ COOH	R-CHO	~ 75	94
R-CHO	R-CH ₂ OH	~ 95	94
R-CHO	R-CH=NOH	~ 95	94
R-CH=NOH	R-C≡N	~ 65	98

× Calculated from radiochemical purity and total radioactivity of reaction product (Initial radiochemical purity of DL-(side chain-3-¹⁴C)-tryptophan 97%).

dropwise under constant stirring until the final pH of the aqueous phase remains constant at 8.5. The benzene phase is pipetted off, dried on sodium sulphate and reduced by rotary film evaporation to a convenient volume for preparative thin layer chromatography (solvent system: chloroform/methanol 96/4, Rf 0.48), on pre-coated silica gel 60 sheets, layer thickness 0,2 mm (Merck). Indole-3-acetonitrile is eluted from the chromatogram with dried benzene and stored at 4°C.

Identity of compounds has been confirmed by co-chromatography with the authentic commercially available unlabelled compounds in different thin layer chromatography systems (Table), by their UV spectra in ethanol and by their colour reaction with Ehmann's reagent (6). Purity was determined by liquid scintillation counting after thin layer chromatography of the reaction products (5). All products have been stored in benzene at 4°C for more than 3 month without decrease in purity.

Rf-values of synthesized compounds chromatographed on silica gel thin layer (200 µm) plates in different solvent systems.

		Indole-3- acetaldehyde	Indole-3- ethanol	Indole-3- acetaldoxime	Indole-3- acetonitrile
Chloroform	96				
Methanol	4	0.35	0.23	0.19	0.48

Benzene	9				
Acetone	1	0.29	0.12	--	0.34

Benzene	7				
Ethylacetate	1	--	--	0.26	--

Note: The volume given for preparation I - IV should not be further reduced but upscaling is possible as long as the volume ratios aqueous phase/benzene phase are maintained constant and thorough mixing is assured.

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