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Note

Separation of 2-amino-3*H*-phenoxazin-3-one impurity from *o*-aminophenol*

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o-Aminophenol (OAP) is an important intermediate for dyes, drugs and pesticides, and for the last two applications it must be highly pure. For use in the production of pesticides such as phosalone, the content of phenoxazone-type coloured impurities in OAP should be lower than 0.5%, otherwise the purity and yields in subsequent stages are affected. A spectrophotometric method for the determination of phenoxazone impurities in commercial OAP has been reported by us[1]. The presence of phenoxazone ring system in dyes and biologically active compounds such as actinomycins has created considerable interest in the synthesis and chemistry of phenoxazones[2–4]. The isolation of aminophenoxazone derivatives from microorganisms, insects, mammalian tissues and plants has been reported[5–8].

The identification of 2-amino-3*H*-phenoxazin-3-one (APZ) as the product of the oxidation of OAP with hydrogen peroxide in the presence of chromium(VI) has been reported[9]. However, so far no such phenoxazones have been isolated as impurities from commercial OAP obtained by catalytic hydrogenation of *o*-nitrophenol (ONP). We detected the presence of trace amounts of APZ by high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC). Different solvents such as chloroform–acetone, chloroform–ethyl acetate, chloroform–acetic acid and ethyl acetate–acetic acid have been suggested[10] for the TLC separation of APZ and its derivatives obtained by the oxidation of OAP and several other substituted phenols. However, R_F values for APZ (isolated from ONP reduction products) using different solvent systems have not been reported. We therefore decided to select a suitable solvent system to improve the separation and isolation of APZ from OAP.

EXPERIMENTAL AND RESULTS

Hydrogenation of ONP

The hydrogenation of ONP (50.0 g) was carried out in a stainless-steel autoclave (Parr type with stirring motor and internal cooling coil) of 2 l capacity using distilled methanol (1 l) as solvent. The pressure was kept at 140 p.s.i.g. and the temperature was maintained at 85°C for 2 h. Raney nickel (Type W-4; 0.5 g) was employed as the catalyst. The course of the reaction was followed by removing

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aliquots at different time intervals through a dip tube under a positive pressure and determining the unreacted ONP volumetrically using titanium(III) chloride[1].

At the end of the reaction the catalyst was allowed to settle and the supernatant solution was siphoned out under positive pressure in a round-bottomed flask containing a stabilizer (hydrazine hydrate, 1.0 ml), through which was passed a continuous stream of nitrogen. One gram of the solid obtained was dissolved in 20.0 ml of concentrated hydrochloric acid. Spectrophotometric assay[1] of the product at 485 nm indicated the presence of 4.0 mg of APZ per gram of the product.

HPLC procedure

HPLC was performed on a Waters Model ALC/GPC-202R401 liquid chromatograph. The system consisted of a UV detector (280 nm) and a dual pen recorder. A 30 cm × 4 mm I.D. μBondapak C_{18} column (Waters Assoc., Milford, MA, U.S.A.) was used. Methanol–water (1:1) was used as the solvent at a flow-rate of 1.5 ml/min and the chart speed was 0.75 in./min. Aliquots (10 μl) were chromatographed and the chromatogram showed two peaks with retention times of 2.9 and 3.6 min corresponding to OAP and APZ, respectively.

TLC analysis

The following solvent systems were tried, using standard conditions on 18 × 4 cm plates coated with 0.5 mm thick layers of silica gel G: (A) benzene–ethyl acetate (9:1); (B) chloroform–ethyl acetate (9:1); (C) chloroform–acetic acid (9:1); (D) chloroform–acetone (9:1); and (E) ethyl acetate–acetic acid (9:1).

Solvents. All solvents were of analytical-reagent grade and were dried and redistilled. They were degassed by refluxing under stirring, followed by cooling in the presence of nitrogen.

Materials. An authentic sample of APZ was prepared by the oxidation of OAP with *p*-benzoquinone[11].

Sample solutions. OAP, APZ and the solid obtained from the reduction of ONP were accurately weighed and dissolved in methanol to the desired volume (concentrations: OAP, 50.0 mg/ml; APZ, 0.1 mg/ml).

Spotting the plates. The sample solutions were spotted on TLC plates using a disposable capillary. The solvent was allowed to migrate to a height of 10 cm above the point of application, then the plate was dried. The spots corresponding to APZ and OAP were easily visible owing to their colours (pale yellow and dark brown, respectively).

Preparative TLC

The R_F values for APZ and OAP using different solvent systems are given in Table I. System C (chloroform–acetic acid, 9:1) afforded the best separation, giving a separation distance of at least 41 mm between the inner edges of the bands of APZ and OAP. Chromatograms run with systems A, B and D showed a tendency to form elongated and diffuse spots, especially that of OAP.

o-Aminophenol (2.0 g) obtained from the hydrogenation mixture dissolved in 40.0 ml methanol was spotted on each of 40 glass plates (20 × 20 cm); the spotting on each plate was completed within 5 min and the plates were further dried for 5 min only. The development was carried out in a glass jar (25.5 × 12 × 25 cm) at room

TABLE I

R_F VALUES OF OAP AND APZ IN DIFFERENT SOLVENTS

R_F values (× 100) are reported as the means of triplicate determinations. Plates were developed in an iodine chamber.

Compound	*Solvent system*				
	A	*B*	*C*	*D*	*E*
OAP (pure)	25	12	9	16	32
OAP (from reduction mixture)	25	12	9	16	32
APZ	29	25	73	38	68

temperature. The development chamber was saturated with nitrogen and the solvents used for development were degassed. Ascending development to a height of 10 cm was completed in 12 min. The bands corresponding to APZ and OAP were easily visible owing to their colours and were scraped off the plate into individual small glass columns fitted with sintered-glass discs. The APZ and OAP were extracted from the adsorbent with ten 5-ml portions of degassed methanol. The solvent was removed by evaporation under nitrogen and the solids obtained were weighed. This method of separation yielded 7.8 mg of APZ (95 % recovery) from OAP. The product APZ had HPLC retention time, IR spectrum and melting point identical with those of an authentic sample and its elemental analysis was in agreement with its structure [ν (Nujol) 3250, 3300, 1653, 1600, 1470, 1389, 1274, 1219 and 1176 cm^{-1}; m.p. 249°C, lit.[11] 249°].

Fig. 1 shows an example of the chromatographic distribution of APZ and OAP.

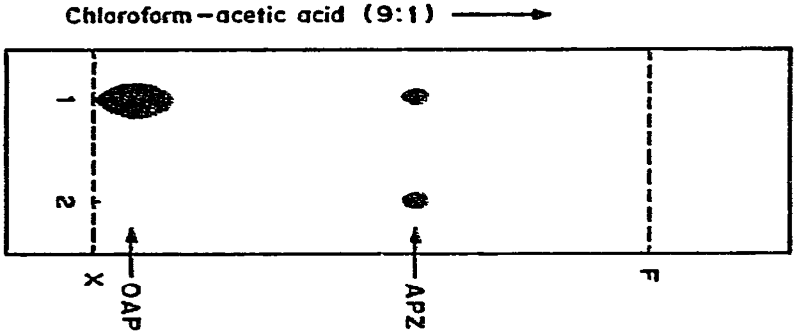

Fig. 1. Thin-layer chromatogram developed with chloroform–acetic acid (9:1). X = origin; F = solvent front. Lanes: 1 = reduction product (OAP); 2 = authentic sample of APZ.

Similar results were obtained when considerably larger volumes of sample (preparative TLC) were spotted. This rapid and one-step elution technique for the separation of APZ enables the amount of APZ formed in OAP to be measured quantitatively and serves as a quality control test for OAP obtained by the hydrogenation of ONP.

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