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Note

Thin-layer chromatographic estimation of 2,4-xyleneol in 2,5-xyleneol

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2,5-Xyleneol is the starting material in synthesis of gemfibrozil, 2,2-dimethyl-5-(2,5-xylyloxy)valeric acid. Studies on the purity of this investigational hypolipemic agent showed the presence of small amounts of its 2,4-xylyloxy isomer, a process contaminant from 2,4-xyleneol in the starting material. Although a rather tedious and lengthy gas chromatographic method was developed for detection and estimation of the isomer in the experimental drug, estimation of the 2,4-xyleneol content of the 2,5-xyleneol used in the synthesis was considered to be most definitive.

Attempts to purify 2,5-xyleneol by crystallization and to separate the 2,5- and 2,4-isomers by gas-liquid chromatographic, high-performance liquid chromatographic and thin-layer chromatographic (TLC) methods were unsuccessful owing to the closely similar physical properties of the isomers. Crump separated *p*-nitrophenylazo derivatives of isomeric phenols by paper partition chromatography¹ and by TLC on alkali-impregnated plates². An operationally simpler TLC system is described here, wherein the azo dye derivatives are made by coupling the xyleneols with commercially available *p*-nitrobenzenediazonium fluoborate. As expected, 2,4-xyleneol couples exclusively at the 6-position, and the predominant product for 2,5-xyleneol is the 4-azo dye. A description of the TLC method and evidence obtained in characterization of the reaction products are presented below.

EXPERIMENTAL AND RESULTS

Determination of 2,4-xyleneol in 2,5-xyleneol

Weigh 25 mg of the 2,5-xyleneol under test and transfer it to a 15-ml centrifuge tube. Dissolve 2,4-xyleneol in 0.1 *M* sodium hydroxide to obtain a concentration of *ca.* 0.5 mg per ml, and transfer 0.05 ml (25 μ g) and 0.5 ml (250 μ g) to similar tubes. Add 5 ml of 0.1 *N* sodium hydroxide to each tube and mix, then add 2 ml of water and 50 mg of *p*-nitrobenzenediazonium fluoborate and mix on a Vortex mixer for 2–3 min. Add 0.75 ml of 1 *N* hydrochloric acid, then extract with 3 ml of hexane-chloroform (7:3), with shaking for 5 min; centrifuge, and collect the top layer. Apply 20 μ l of each solution *ca.* 2.5 cm from one edge of a TLC plate (20 \times 20 cm) coated with a 0.25-mm layer of silica gel GF, and develop the plate in the hexane-chloroform (7:3) until the solvent front has ascended *ca.* 15 cm, then air-dry the plate, and spray it with 1 *M* methanolic tetrabutylammonium hydroxide. Estimate the amount of

2,4-xylenol in the lane from the 2,5-xylenol by comparison with the two standards (0.1% and 1%). The azo dye from 2,4-xylenol has an R_F of *ca.* 0.3, and is well resolved from the dyes from 2,5-xylenol, which show R_F values from the origin to less than 0.2. (Development of the chromatogram can be followed by the orange spots of the azo dyes; these are changed to purple and intensified when treated with alkali.)

Characterization of azo dyes

6-(4-Nitrophenylazo)-2,4-xylenol was prepared by adding 3 g of *p*-nitrobenzenediazonium fluoborate to a solution of 2 g of 2,4-xylenol in 100 ml of 0.1 *M* sodium hydroxide and stirring for 5 min. The solution was acidified by dropwise addition of concentrated hydrochloric acid and extracted with 50 ml of chloroform, and the extract was washed with three 25-ml portions of water and then evaporated to dryness. The residue, which gave essentially one spot on TLC, was recrystallized once from aqueous acetic acid and twice from glacial acetic acid to yield dark pink crystals, which were washed with cold water and dried in a vacuum oven at 90°. Elemental analysis, mass spectra and nuclear magnetic resonance (NMR) studies gave results consistent with the structure assigned.

Calculated for $C_{14}H_{13}N_3O_3$: C = 61.99, H = 4.79, N = 15.49

Found: C = 61.27, H = 4.93, N = 15.46

The mass spectrum showed a molecular ion at m/e 271 (the molecular weight) and additional peaks for fragments m/e 149, 121, 91 and 77. NMR in deuteriochloroform showed peaks at 2.28 and 2.36 ppm (diagnostic of two aromatic methyls), at 7.1 and 7.55 ppm (indicating protons at the 3- and 5-positions on the xylenol ring), at 7.87 to 8.37 ppm (for the four protons on the 4-nitrophenyl moiety) and at 12.83 ppm for the proton on the xylenol -OH group.

4-(4-Nitrophenylazo)-2,5-xylenol was prepared similarly, except that the reaction was allowed to proceed for 25 min. The residue was impure by TLC. It was purified by introducing 150 mg of it, in chloroform, into a glass column (37 cm \times 3.2 cm I.D.) packed with SilicAR®-CC-4 (Mallinckrodt, St. Louis, Mo., U.S.A.) slurried in toluene, eluting with toluene and monitoring the fractions visually. The major component was chromatographed on a similar column, using chloroform for elution. The eluate was evaporated to dryness, and the residue was recrystallized from aqueous methanol to give brown crystals, which melted at 225–227°. Elemental analysis, mass spectra and NMR spectra were consistent with the assigned structure.

Calculated for $C_{14}H_{13}N_3O_3$: C = 61.99, H = 4.79, N = 15.49

Found: C = 61.51, H = 4.87, N = 15.46

The mass spectrum showed a molecular ion at m/e 271 and was similar to that obtained for the azo dye from 2,4-xylenol. NMR in deuteriochloroform showed peaks at 2.17 and 2.60 ppm (for the two aromatic methyls), a peak at 4.98 ppm (for the -OH proton), peaks at 6.64 and 7.50 ppm (diagnostic of the protons at the 6- and 3-positions of the xylenol ring) and at 7.78 to 8.28 ppm (for the four protons of the 4-nitrophenyl moiety).

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REFERENCES

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