GLC Determination of Degradation of Two Related Amine Uptake Inhibitors

REX W. SOUTER ˣ **and ALAN DINNER**

Abstract □ A convenient GLC method was developed to assay the chemically related CNS agents *dl*-3-(*p*-trifluoromethylphenoxy)-*N*-methyl-3-phenylpropylamine (I*a*) and *dl*-3-(*o*-methoxyphenoxy)-*N*-methyl-3-phenylpropylamine (I*b*). Subsequently, this GLC method was used to monitor the stability of I*a* and I*b* in acidic and basic media. These CNS agents were stable in basic solution but labile under acid conditions, and the degradations of both compounds approximated pseudo-first-order kinetics. Products formed during the acid hydrolysis of I*a* and I*b* were identified and were shown not to interfere in the GLC assays of the parent ethers.

Keyphrases □ GLC—stability determination, 3-phenylpropylamines, CNS agents □ CNS agents—3-phenylpropylamines, GLC stability determination

After the synthesis of a potential new drug, a suitable analytical assay procedure is needed for stability studies. Ideally, this method should be facile, able to detect the drug at low levels, and valid in the presence of chemical degradation products of the drug.

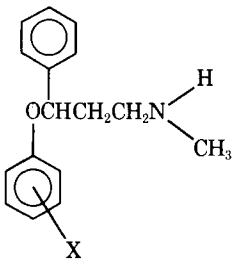
This paper reports a method for the assays of the selective serotonin uptake inhibitor (1-3) *dl*-3-(*p*-trifluoromethylphenoxy)-*N*-methyl-3-phenylpropylamine (I*a*) and the norepinephrine uptake inhibitor (2) *dl*-3-(*o*-methoxyphenoxy)-*N*-methyl-3-phenylpropylamine (I*b*). Both compounds are currently under study as inhibitors of uptake into monoaminergic neurons in brain.

EXPERIMENTAL

Reagents—The solvents used were all of analytical reagent quality. The 99% *n*-pentacosane[1] used as internal standard, the OV-225 liquid phase[2], and the acid-washed, dimethyldichlorosilane-treated Chromosorb G[3] were all purchased materials.

Apparatus—A high-precision gas chromatograph was used[4]. Screw-capped vials[5] with septums[6] were used for all sample extractions.

Chromatographic Conditions—The column was a 0.9-m × 0.83-cm (3-ft × 0.25-in.) o.d. acid-washed, dimethyldichlorosilane-treated glass U-tube packed with 4% OV-225 on 80–100-mesh Chromosorb G. The oven was operated at 200° for studies of I*a* and at 215° for studies of I*b*. Helium was used as the carrier gas (after passing through a drying trap packed with 5A molecular sieves) at an approximate flow rate of 60 ml/min.



I*a*: X = *p*-CF_3
I*b*: X = *o*-OCH_3

Procedure—Peak areas were calculated using an on-line calculation program from the expanded RTE 2100 computer system[4]. Area ratios of sample–internal standard usually agreed within 1% for replicate injections.

For the degradation studies, a known amount (150–300 mg) of I*a* or I*b* as the hydrochloride salt was added to 100 ml of refluxing 0.1 *N* HCl. At various time intervals, aliquots of solution were removed and cooled in ice water. A 2-ml sample of each cooled aliquot was pipetted into the screw-capped septum vial. To this vial was added 7.4 *N* NH_4OH (to bring the pH to 7.5–8), and the solutions were mixed. Finally, 2.0 ml of a chloroform solution of the internal standard *n*-pentacosane (0.5–0.6 mg/ml) was added, the vial was capped, and the mixture was shaken for 2 min.

The two layers were allowed to separate, and then samples of the chloroform portion were injected into the GLC column. Plots of log [ether] *versus* time were constructed using a least-squares program available through a computer[7], and the slopes of the resulting straight lines were used to calculate the pseudo-first-order rate constants. Reactions were followed for at least two half-lives (C and D in Fig. 1). NMR spectra were obtained[8] using tetramethylsilane as the internal reference. Mass spectra were obtained on a gas chromatograph–mass spectrometer[9] unit at 70 ev.

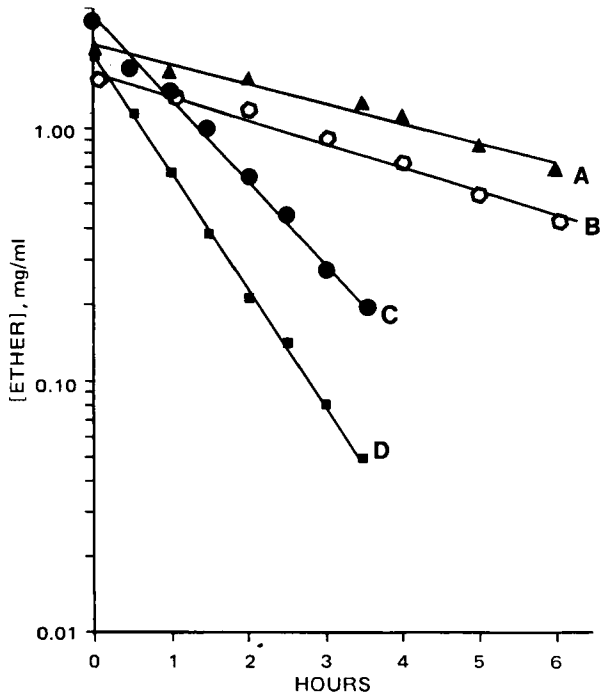
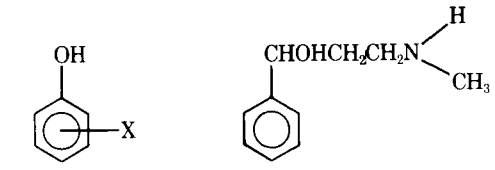


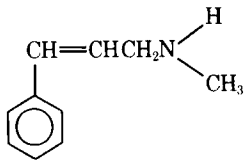
Figure 1—*Kinetic plots for acid degradation of* I*a and* I*b. Key: A,* I*a, initial measured concentration = 2.03 mg/ml, slope = −0.078; B,* I*a, initial measured concentration = 1.50 mg/ml, slope = −0.097; C,* I*b, initial measured concentration = 2.63 mg/ml, slope = −0.33; and D,* I*b, initial measured concentration = 1.97 mg/ml, slope = −0.46.*

[1] Lachat Chemicals, Mequon, Wis.
[2] Ohio Valley Specialty Chemicals, Marietta, Ohio.
[3] Johns-Manville, Denver, Colo.
[4] Model F&M 402, Hewlett-Packard, Avondale, Pa.
[5] No. 13028, Pierce Chemical Co., Rockford, Ill.
[6] Lined with Teflon, Canton Biomedical Products, Boulder, Colo.
[7] DEC-10, Digital Equipment Corp., Marlborough, Me.
[8] Varian T-60A, Varian Instrument Division, Palo Alto, Calif.
[9] LKB 9000S, LKB Instruments, Rockville, Md.



IIa: X = p-CF_3
IIb: X = o-OCH_3

III



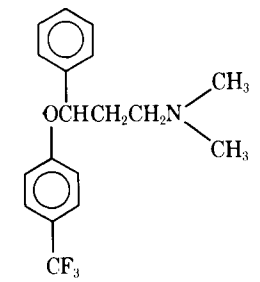
IV

Decomposition of *dl*-3-(*p*-Trifluoromethylphenoxy)-*N*-methyl-3-phenylpropylamine (Ia)—A solution of 300 mg (1 mmole) of Ia in 100 ml of 0.1 *N* aqueous hydrochloric acid was allowed to reflux for 24 hr. The solution was cooled, adjusted to pH 8 with 7.4 *N* NH_4OH, and then extracted with three 50-ml portions of chloroform. The chloroform layers were combined, dried over anhydrous magnesium sulfate, and finally concentrated on a rotary evaporator at 25°.

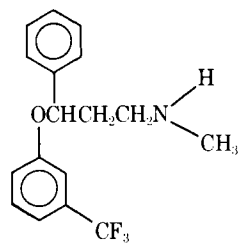
A portion of the concentrated chloroform solution was streaked onto a 500-μm TLC plate[10], and the plate was developed using methanol–acetic acid (90:10) as the eluant. Three zones were observed under 254-nm UV light (R_f 0.83, 0.65, and 0.55). The zones were scraped from the plate and eluted with chloroform, and the resulting solutions were evaporated. The fastest-moving zone was identified as *p*-trifluoromethylphenol (IIa); NMR (carbon tetrachloride): δ 7.65 (d, 2H, J = 8 Hz, aromatics), 6.9 (d, 2H, J = 8 Hz, aromatics), and 6.3 (s, 1H, OH); mass spectrum molecular ion at *m/e* 162, calculated for $C_7H_5F_3O$, 162; prominent fragmentation peak at *m/e* 143 ($p^+ - F$). TLC comparison with an authentic sample gave the same R_f values in two different solvent systems.

The middle zone was identified *via* IR comparison as unreacted starting material. The slowest-moving zone was identified as *N*-methyl-3-phenyl-3-hydroxypropylamine (III); NMR (deuterated methanol): δ 7.4 (s, 5H, aromatics), 4.9 (t, 1H, J = 6 Hz, benzylic H), 3.1 (t, 2H, J = 6 Hz, NCH_2—), 2.6 (s, 3H, NCH_3), and 2.1 (q, 2H, J = 6 Hz, $CHCH_2$—); mass spectrum molecular ion at *m/e* 165, calculated for $C_{10}H_{15}NO$, 165; major fragment at *m/e* 147 ($M^+ - H_2O$).

Decomposition of *dl*-3-(*o*-Methoxyphenoxy)-*N*-methyl-3-phenylpropylamine (Ib)—This material was degraded and worked up in a manner similar to that of Ia. Development of the thin-layer chromatogram revealed the presence of three zones (R_f 0.69, 0.65, and 0.55). The fastest-moving zone was shown to be guaiacol (*o*-methoxyphenol, IIb) *via* IR comparison with authentic material. The middle zone was unreacted starting material (IR comparison), and the slowest-moving zone was identified (as for Ia) as III.



V



VI

[10] Silica gel F-254, EM Labs., Elmsford, N.Y.

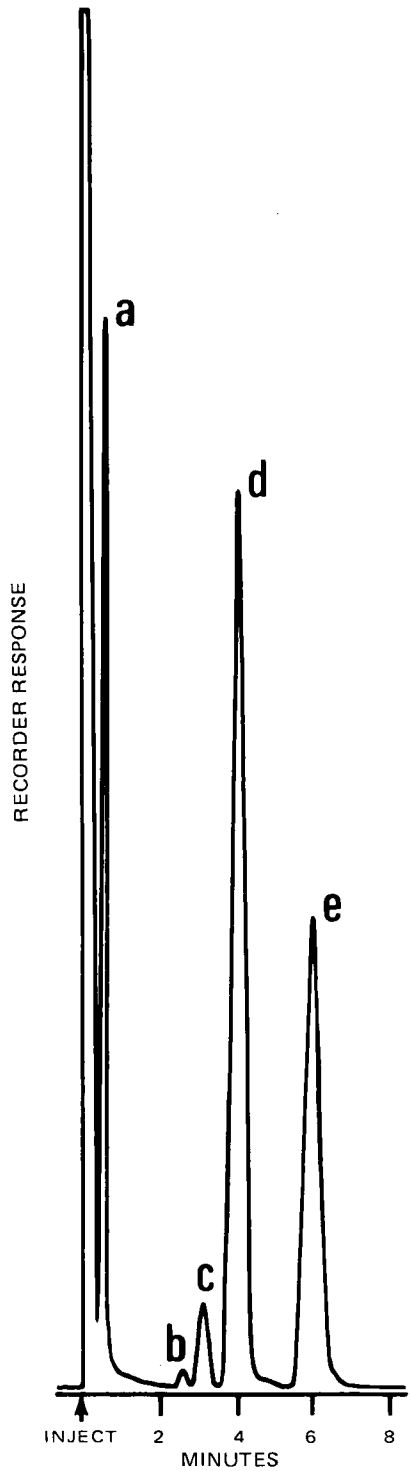


Figure 2—*Typical gas chromatogram of partially degraded Ia. Key: a,* p-*trifluoromethylphenol; b, V; c, VI; d,* Ia; *and e,* n-*pentacosane.*

RESULTS AND DISCUSSION

Calibration curves for Ia and Ib were obtained, and they re-

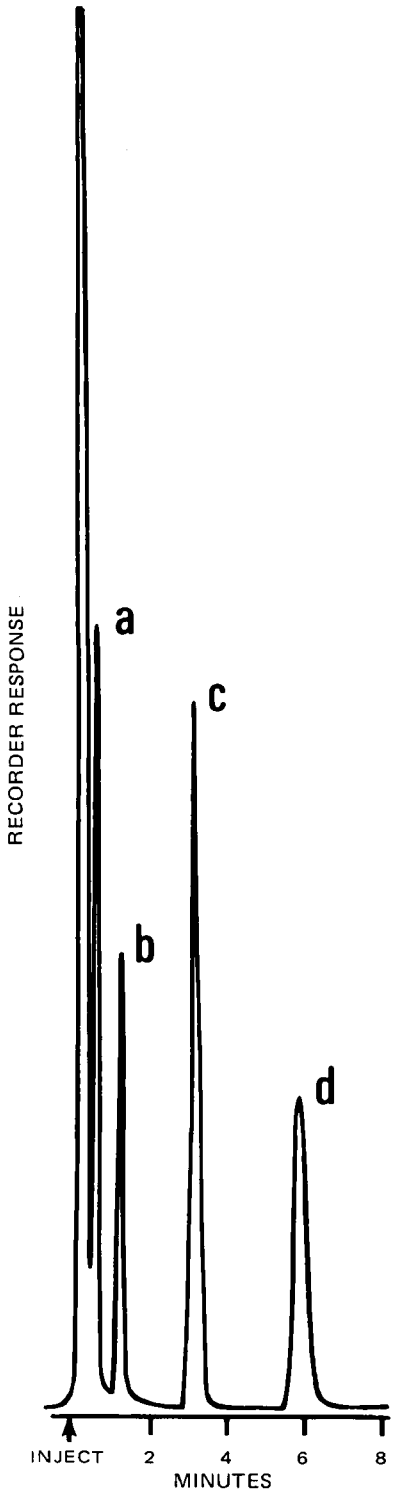


Figure 3—*Typical gas chromatogram of partially degraded Ib. Key: a, IIb; b, IV; c, n-pentacosane; and d, Ib.*

vealed a linear relationship between the peak area of compound–peak area of internal standard and the concentration of sample injected between 1.0×10^{-2} and 2.4 μg/μl. Quantities as low as 10 ng were detectable for either compound.

The kinetics of the acidic degradation of solutions of ethers Ia and Ib at two different starting concentrations are shown in Fig. 1. The degradations of both compounds approximate pseudo-first-order kinetics, with a k value of 0.20 hr^{-1} for Ia and of 0.90 hr^{-1} for Ib. Although these conditions are generally too mild for the cleavage of either aryl alkyl (4, 5) or dialkyl ethers (6, 7), the hydrolysis of aryl benzyl ethers is quite facile (8, 9). Benzyl phenyl ether itself has been cleaved in various solvents with hydrobromic acid at room temperature (10).

Literature reports of the solvolyses of other benzyl phenyl ethers (9, 10) suggest that the cleavage rates of the two compounds examined were not unusual.

The products of the acid-catalyzed cleavage of Ia were *p*-trifluoromethylphenol[11] (IIa) and *N*-methyl-3-phenyl-3-hydroxypropylamine (III), while Ib yielded *o*-methoxyphenol (IIb) and III. As expected, no benzyl halide was found since it would be hydrolyzed immediately under the reaction conditions (11) to the observed benzyl alcohol; no *O*-demethylation of Ib was noted.

The NMR, IR, and mass spectral data were consistent with the proposed structures. A combined GLC–mass spectral analysis of the product mixture from either Ia or Ib also revealed a small amount of material (1%) at m/e 147. The major fragments at m/e 146 and 117 $(C_6H_5CH{=}CHCH_2)^+$ strongly suggest IV as the likely structure. Injection of purified III into the gas chromatograph revealed no decomposition of IV. Hence, IV is generated from the acid-catalyzed dehydration of III in the reaction mixture.

Figures 2 and 3 represent typical chromatograms of partially degraded samples of Ia and Ib. Clearly, the degradation products do not interfere with the starting material or with the internal standard. In Fig. 2, peaks b and c represent impurities present in the Ia used. The identities of b and c were determined by use of GLC–mass spectrometry and by comparisons of chromatographic retention times with authentic samples. Peak b is 3-(*p*-trifluoromethylphenoxy)-*N*,*N*-dimethyl-3-phenylpropylamine (V), and peak c is 3-(*m*-trifluoromethylphenoxy)-*N*-methyl-3-phenylpropylamine (VI).

Attempted basic degradation of either Ia or Ib with 0.1 *N* aqueous sodium hydroxide at 100° resulted in complete recovery of starting material after 3 days. In general, alkali metals are required for the basic cleavage of benzyl aryl ethers (12).

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[11] This material was shown to be a metabolite of Ia in the rat; C. J. Parli, unpublished results.