Table II—Spectral Data for Diaryl-4,5,6,7-tetrahydroimidazo-[4,5-c]pyridines

Compound	IR Spectrum (KBr): ν_{max}, cm^{-1}	^{1}H-NMR Spectrum (deuterodimethylsulfoxide), ppm
II	3100–2800 (bd), 1610, 750, and 695	2.5 (t, 2H, C-4, CH_2), 2.7 (t, 2H, C-5, CH_2), and 7.16–7.4 (m, 11H, Ar, C-2)
III	3250–2850 (bd), 1610, 775, and 745	2,6 (t, 2H, C-4, CH_2), 2.9 (t, 2H, C-5, CH_2), 7.2 (m, 2H, Ar), 7.6 (5H, Ar, C-2), and 8.3 (2H, Ar)
IV	3100–2600 (bd), 1610, 750, and 695	2.16 (t, 2H, C-4, CH_2), 2.7 (t, 2H, C-5, CH_2), 2.9 (d, 2H, CH_2), 3.4 (d, 2H, CH_2), 7.2 (m, 10H, Ar), and 7.6 (S, 1H, C-2)
V	3200–2850 (bd), 1620 and 745	3.3 (t, 2H, C-4, CH_2), 3.75 (t, 2H, C-5, CH_2), 8.06 (S, 1H, C-2), and 7.6 (m, 8H, Ar)
VI	3200–2800 (bd), 1620, 1580, 790, and 755	2.6 (t, 2H, C-4, CH_2), 3.1 (t, 2H, C-5, CH_2), 6.8 (m, 2H, Ar), 7.8 (m, 3H, Ar, C-2), and 8.3 (m, 2H, Ar)

RESULTS AND DISCUSSIONS

The condensation of histamine free base (I) and a variety of aromatic and heteromatic ketones has given rise to a series of novel 4,5,6,7-tetrahydroimidazo-[4,5-c]pyridines. The proposed structures are supported by pertinent spectral data and are consistent with ring closure at C-5 of the imidazole ring with concomitant loss of the characteristic aromatic proton at 6.9 ppm.

A preliminary gross behavioral evaluation of each of these compounds in mice at three different dose levels (33, 54, and 66 mg/kg) has shown that the dipyridyl compound (III) exhibits a marked degree of convulsant activity. None of the other compounds were found to produce convulsions at the same dose levels. Research is currently in progress to establish the possible mode of action of this novel heterocyclic system.

EXPERIMENTAL[1]

All syntheses were carried out by dissolving the appropriate ketone in *n*-butanol and treating the stirred solution with one equivalent of histamine-free base in *n*-butanol. The solutions were refluxed and monitored using TLC (cellulose; *n*-butanol–acetic acid–water, 4:1:1) until no further ketone could be detected. Removal of the solvent under reduced pressure followed by repeated dilutions with methanol and then with acetone caused the precipitation of the crude free bases of the condensation products. The resulting free bases were converted to their corresponding hydrochlorides which could be recrystallized from ethanol–acetone.

REFERENCES

(1) D. Heyl, E. Luz, S. Harris, and K. Folkess, *J. Am. Chem. Soc.*, **70**, 3669 (1943).

(2) F. Stocker, M. Fordice, J. Larson, and T. H. Thorestenson, *J. Org. Chem.*, **31**, 2380 (1966).

(3) G. Habermehl and W. Ecsy, *Heterocycles*, **5**, 127 (1976).

ACKNOWLEDGMENTS

The authors acknowledge the financial support of Old Dominion University.

[1] Melting points for all compounds are uncorrected and were determined on a Thomas-Hoover melting point apparatus. IR spectra were recorded on a Perkin-Elmer 137 spectrometer. NMR spectra were recorded on a Varian T-60 spectrometer in deuterodimethylsulfoxide using tetramethylsilane as the internal standard. All C, H, and N analyses were performed by the M-H-W Laboratories, Phoenix, Ariz.

Quality Control of Phenylbutazone II: Analysis of Phenylbutazone and Its Decomposition Products in Drugs by High-Pressure Liquid Chromatography

H. FABRE ˣ, B. MANDROU, and H. EDDINE

Received June 2, 1980, from the *Laboratoire de Chimie Analytique, Faculté de Pharmacie, 34060 Montpellier Cedex, France.* Accepted for publication June 17, 1981.

Abstract □ A rapid, sensitive, accurate, and reproducible procedure for the simultaneous separation and determination of phenylbutazone and three major degradation products is proposed using reversed-phase high-pressure liquid chromatography and UV detection. The method is ~20 times more sensitive than TLC and allows an accurate determination of degradation products without decomposition during the analysis.

Keyphrases □ Phenylbutazone—analysis by high-pressure liquid chromatography, decomposition products □ High-pressure liquid chromatography—analysis of phenylbutazone and its decomposition products □ Degradation—phenylbutazone, analysis by high-pressure liquid chromatography

An earlier report (1) outlined the difficulties relative to the establishment of an analytical procedure to detect the intermediate products of oxidation and hydrolysis of phenylbutazone (I). These products are 4-hydroxyphenylbutazone (II), *N*-(2-carboxycaproyl)hydrazobenzene (III), and *N*-(2-carboxy-2-hydroxycaproyl)hydrazobenzene (IV). GLC and TLC are not reliable procedures to monitor the stability of I, because artifacts are formed. TLC can only be used with special precautions as a qualitative and quantitative test to determine I–IV (1).

Reversed-phase high-pressure liquid chromatography (HPLC) is often used in stability studies because it is selective and rapid, and it is particularly recommended when the compound is easily oxidized and when extraction procedures may be degradative. No sample preparation is required for aqueous solutions and reversed-phase HPLC is especially attractive for injections of I because I undergoes decomposition in aqueous medium. Re-

0022-3549/82/0100-0120$01.00/0

Table I—Retention Time Values for Phenylbutazone and Degradation Compounds

Compound	Retention Time, sec	Retention Time Relative to I	Retention Time Relative to V
I	509	1.00	1.58
II	824	1.62	2.55
III	242	0.48	0.75
IV	138	0.27	0.43
V	323	0.64	1.00
lidocaine[a]	562	1.10	1.74

[a] Evidenced with 1 g/liter of lidocaine solution.

Table II—Minimum Amount Detectable and Sensitivity for I–V at λ 237–267 nm

Compound	Minimum Amount Detectable, μg		Sensitivity	
	λ = 237 nm	λ = 267 nm	λ = 237 nm	λ = 267 nm
I	8.9×10^{-3}	6.5×10^{-3}	15,225	19,000
II	6.5×10^{-3}	40.0×10^{-3}	19,951	4200
III	2.0×10^{-3}	7.8×10^{-3}	20,600	6000
IV	1.8×10^{-3}	9.4×10^{-3}	19,000	3000
V	3.7×10^{-3}	—	12,770	2043

Table III—Recovery Data from Laboratory Prepared Injections

	Amount Added, mg	Amount Found, mg	Relative Error, %
Placebo	—	—	—
Placebo +I	1000.00	1013.00	1.30
Placebo +I	1000.00	1009.48	0.95
+II	5.00	5.14	2.80
+III	5.00	5.03	0.60
+IV	5.00	5.13	2.60
Placebo +I	1000.00	1030.70	3.07
+II	20.00	19.02	−4.90
+III	20.00	19.99	−0.05
+IV	20.00	20.08	0.40
Placebo +I	1000.00	999.99	0.00
+II	30.00	28.92	−3.60
+III	30.00	30.11	0.38
+IV	30.00	29.29	−2.37

versed-phase HPLC was recently reported (2) to determine I and II metabolites in biological fluids, but there are no literature references relative to the determination of I and its main degradation products *in vitro*. The present study reports a rapid, sensitive, and accurate procedure for simultaneously quantitating I–IV, and an application for injections with lidocaine is given.

EXPERIMENTAL

Material and Reagents—A high-pressure liquid chromatograph[1] with a variable-wavelength UV detector[2], a 10-μl automatic loop injection system, an integrator, and a reversed-phase column[3] were used. Compounds I–IV were used as received[4]. Tromethamine, citric acid, and dimethyl phthalate (V) were analytical reagent grade. Acetonitrile was HPLC reagent grade. Injections (1 g of phenylbutazone, and 50 mg of lidocaine/5 ml of aqueous solvent) were commercial formulations[5]. Distilled water was filtered through a 0.45-μm filter[6]. The *mobile phase* was

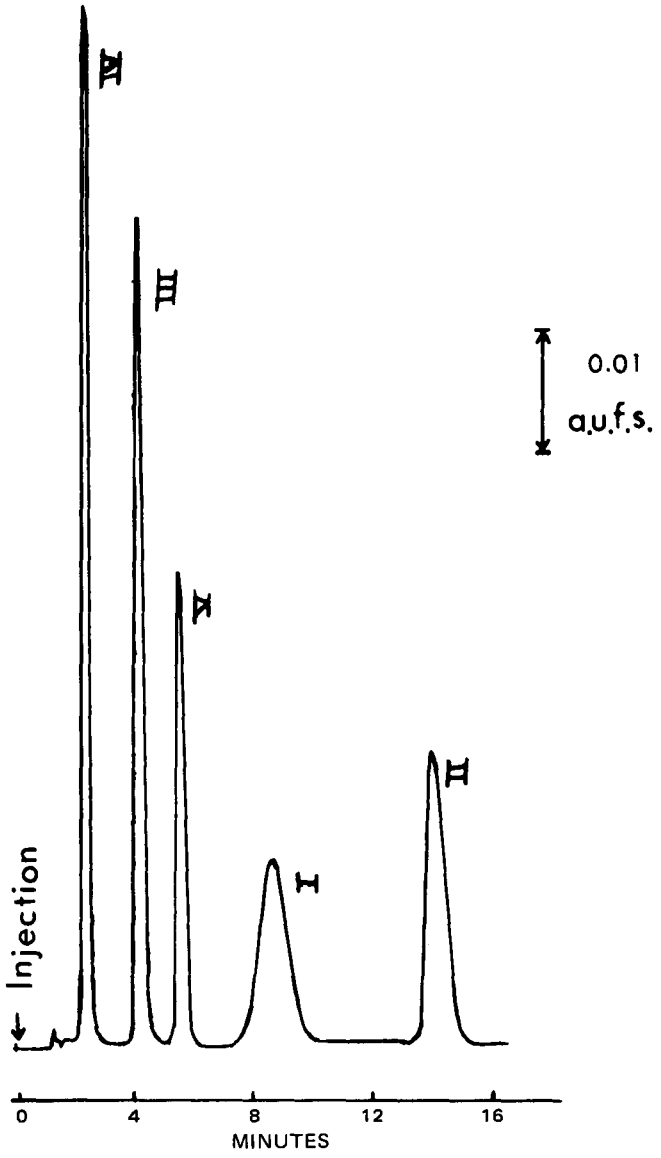

Figure 1—*Chromatogram of a standard solution of I (65.0 μg/ml), II (52.5 μg/ml), III (58.0 μg/ml), IV (68.0 μg/ml), and V (59.6 μg/ml). The mobile phase was 0.1 M tromethamine citrate buffer (pH 5.25)–acetonitrile (60:40); flow was 1.8 ml/min; and pressure was 83 bars. Detector sensitivity 0.1 aufs; chart recorder speed was 0.5 cm/min; and λ = 237 nm.*

0.1 *M* tromethamine citrate buffer[7] (pH 5.25)–acetonitrile (60:40); the mixture was previously degassed.

Standard Solutions—A mixed stock standard solution was prepared in the mobile phase, using I–IV (1000 μg of each per ml). This solution was suitably diluted and an equal volume of internal standard solution (100 μg/ml of V in the mobile phase) was added to give a concentration of 12.5–125 μg of each compound/ml.

Test Solutions—Recoveries of I–IV were carried out on laboratory prepared injections (analogous to commercial formulations) with and without added amounts of II, III, and IV (0.5, 2, and 3.0%, respectively, with respect to I). These solutions and the commercial formulations were suitably diluted in the mobile phase and internal standard was added to match the calibration graph.

Chromatography—Duplicate injections (10 μl) of each solution were injected under the following isocratic conditions: flow rate, 1.8 ml/min;

[1] Spectra-Physics SP 8000.
[2] Schoeffel model SF 770.
[3] 5-μm RP 18, 25-cm length × 4.6-mm diameter, laboratory-made column.
[4] Courtesy of Geigy Laboratories, Basel, Switzerland.
[5] Geigy Laboratories. Injections did not have the same reference numbers as those previously used (1).
[6] Millipore.

[7] Made by dissolving 24.2 g of tromethamine in 70 ml of water, adjusting the pH to 4.7 with citric acid, and diluting to 100 ml with water. The pH 5.25 buffer solution was prepared with a 5-ml aliquot diluted to 100 ml.

pressure, 83 ± 2 bars; chart speed, 0.5 cm/min, detector sensitivity, 0.04–0.1 aufs; and oven temperature, 20°. The chromatograms were recorded at 237 nm.

RESULTS AND DISCUSSION

The maximum absorbance wavelengths in the mobile phase were 267 (I) and 237 (II–IV). A weak absorbance of V was observed at 267 nm; 237 nm was most suitable for the simultaneous determination of I–V. In injections, lidocaine did not absorb at 237 and 267 nm at the concentration used (125 μg/ml) for the determination of II–IV.

A chromatogram of a standard solution is shown in Fig. 1 and Table I lists the retention times of each compound.

Figure 1 and Table I show a good resolution of each compound. The resolution obtained was always better than 1 for I–V.

A calibration graph was plotted of peak area ratio of the solute to the internal standard against solute concentration. The calibration graph was linear in the concentration range studied and went through the origin, using peak areas as well as peak heights. The correlation coefficients of the linear regression analysis were always better than 0.999 using peak areas as well as peak heights.

The minimum amount detectable, defined as the amount (in micrograms) that gives a peak height equal to twice the background, and the sensitivity, defined as the change in area value (measured at the maximum detector sensitivity) resulting from a concentration change of one unit (milligrams per liter) are given in Table II.

The repeatability tested by five replicates and evaluated by the coefficient of variation was 0.978 (I), 1.064 (II), 1.210 (III), and 1.510% (IV). The average data of duplicate assays for recovery studies on laboratory prepared injections with and without added amounts of II–IV are given in Table III.

A comparative separation of I–IV was carried out on a silica column[8]. With hexane–chloroform–methanol–formic acid (400:200:20:1) as a mobile phase, retention times were ~4 (I), 8 (II), 12 (III), 17 (IV), and 25 min for benzenesulfonamide used as an internal standard. Reversed-phase HPLC was faster (14 min) and more convenient than normal HPLC. The insignificant amounts of II–IV (probably present in the reference drug) detected by injecting 10 μl of a concentrated solution of I (2.5 g/liter) and the accuracy obtained for the determination of trace II–IV reveal that reversed-phase HPLC allows the determination of I–IV without decomposition during analysis.

With GLC, artifact peaks were observed when degradation products were present, and it is suggested (3) that the breakdown of the degradates occurs at the injection port maintained at 230°. HPLC is achieved at ambient temperature and prevents this drawback.

With TLC, a significant oxidation of I on the plate was observed during analysis (1). The iron present in the silica coating was responsible (1) for the accelerated air oxidation of I. The absence of degradation with the HPLC procedure is consistent with this hypothesis. The analysis time (13 min), the absence of air exposure, and the presence of citric acid (chelating agent for iron) in the mobile phase (used as an eluting agent and as a solvent in the preparation of solutions) contribute to prevent the degradation of I.

A commercial injection formulation was analyzed by the proposed HPLC procedure. Compound I was 1000.2 mg (100.0% of the labeled strength), III was 14.92 mg (1.49% with respect to theoretical I content), IV was 21.8 mg (2.18% with respect to theoretical I content), and II was not detected. The results are in agreement with other data (4) that shows II is so readily hydrolyzed into IV that it cannot be found in alkaline solution, and the major products of I degradation in injections are III and IV.

Reversed-phase HPLC is a sensitive (20 times more than TLC) and convenient procedure that can be used to evaluate the purity of phenylbutazone and to monitor its stability in pharmaceutical formulations. Because the metabolites of I have been identified as similar to the degradation products *in vitro*, the method is suggested to be applicable to pharmacokinetic studies.

[8] 30 μm Zorbax Sil, 30-cm length.

REFERENCES

(1) H. Fabre and B. Mandrou, *J. Pharm. Sci.*, **70**, 460 (1981), and references therein.

(2) T. Marunaka, T. Shibata, Y. Minami, and Y. Umeno, *J. Chromatogr.*, **183**, 331 (1980).

(3) J. R. Watson, F. Matsui, R. C. Lawrence, and P. M. J. McConnell, *J. Chromatogr.*, **76**, 141 (1973).

(4) D. V. C. Awang, A. Vincent, and F. Matsui, *J. Pharm. Sci.*, **62**, 1673 (1973).

COMMUNICATIONS

Active Conformation of Polycyclic Antidepressants

Keyphrases □ Antidepressants, polycyclic—active conformation □ Conformation, active—of polycyclic antidepressants

To the Editor:

A 300-MHz ^{1}H-NMR study of a series of polycyclic (tetracyclic and pentacyclic) antidepressant agents of the amitriptyline type indicated a preferred conformation in solution in which the alkylamino side chain is folded toward the polycyclic skeleton and its positively charged dimethylammonium ion is oriented above the adjacent aromatic ring.

Amitriptyline (I) and imipramine, prototype tricyclic antidepressant drugs, are potent inhibitors of active reuptake of biogenic amines in nerve endings. The therapeutic effects of these drugs may be related to this activity[1] (1–5). Reuptake inhibition may lead to an accumulation of neurotransmitters, *e.g.*, norepinephrine and serotonin, at the receptor site and to a subsequent increase in activity. The preferred conformation of the tricyclic antidepressant at the biogenic amine uptake jump may be crucial to their antidepressant activity.

Of particular significance is the conformation of the alkylamino side chain *vis-à-vis* the tricyclic skeleton (6, 7). X-ray structure determinations (8–13) indicated that in the crystalline state the side chain is almost fully extended, away from the tricyclic skeleton. However, it was suggested (6, 14) that the active conformation of the alkylamino side chain in polycyclic antidepressants is folded toward the aromatic ring. We present evidence for the amitriptyline-type series in favor of this hypothesis and

[1] For the state of the art of the mode of action of tricyclic antidepressants, see Horn (5).

0022-3549/82/0100-0122$01.00/0