Synthesis of *N*-Pentafluorobenzoyl-S-(−)-prolyl 1-Imidazolide, A New Electron-Capture Sensitive Reagent for Determination of Enantiomeric Composition

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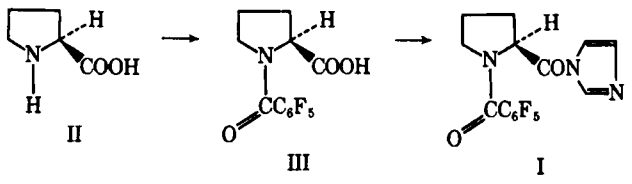
Abstract □ The synthesis of *N*-pentafluorobenzoyl-S-(−)-prolyl 1-imidazolide, a new electron-capture sensitive derivatizing reagent, is described. Its potential usefulness in the detection and quantitative estimation of the enantiomeric composition of amines at nanogram levels is discussed.

Keyphrases □ *N*-Pentafluorobenzoyl-S-(−)-prolyl 1-imidazolide—synthesized as electron-capture sensitive reagent for the GLC determination of enantiomeric composition □ Enantiomers, amines—determination of nanogram levels using *N*-pentafluorobenzoyl-S-(−)-prolyl 1-imidazolide as an electron-capture sensitive derivatizing reagent □ Electron-capture detection, nanogram levels of enantiomeric amines—use of *N*-pentafluorobenzoyl-S-(−)-prolyl 1-imidazolide as a derivatizing reagent

It is well established that derivatization of enantiomers with a chiral reagent yields diastereomers which often can be easily resolved and quantitatively estimated by gas–liquid partition chromatography (1–6). Utilizing this principle, Halpern and Westley (7) introduced the use of *N*-trifluoroacetyl-S-(−)-prolyl chloride to analyze the enantiomeric composition of amino acids. Gordis (8), Gunne (9), and later Wells (10) extended the use of this reagent to the determination of the enantiomeric composition of microgram quantities of amphetamine.

A second type of derivatizing reagent for primary and secondary amines utilizes moieties that are electron-capture sensitive. Such a process may enhance the gas–liquid partition chromatographic detectability of these amines several thousandfold (11). The pentafluorobenzamides of primary and secondary amines afford excellent electron-capture sensitivity (11), in contrast to the corresponding trifluoroacetamides which give almost no electron-capture response (12). For example, the *N*-pentafluorobenzoyl derivative of amphetamine can be detected at 1/6000th the level of the corresponding trifluoroacetamide. In an attempt to combine the electron-capture sensitive properties of the pentafluorobenzoyl moiety with the enantiomeric resolving property of the prolyl moiety, *N*-pentafluorobenzoyl-S-(−)-prolyl 1-imidazolide (I) was synthesized by the following route. S-(−)-Proline (II) was converted to its *N*-pentafluorobenzoyl derivative (III) under Schötten–Baumann conditions. The resulting amido acid was treated with *N,N'*-carbonyldiimidazole to yield the desired 1-imidazolide (I), a high melting, crystalline solid which proved to be stable when stored under anhydrous conditions (Scheme I).



II III I

Scheme I

EXPERIMENTAL

***N*-Pentafluorobenzoyl-S-(−)-proline (III)**—To a stirred suspension of S-(−)-proline (1.0 g., 8.7 mmoles) in 10 ml. water, maintained at 0° by an ice bath, was added dropwise a 10-ml. solution of ice-cold 0.2 *N* NaOH. When dissolution of the amino acid was complete, pentafluorobenzoyl chloride (2.0 g., 8.7 mmoles) was added slowly followed by the periodic addition of 0.2 *N* NaOH over a 2–3-hr. duration to maintain the pH of the reaction mixture at 8.0. After 30 min. of additional stirring in the cold, the reaction mixture was acidified with 0.2 *N* HCl and the resulting mixture was extracted with ether. After drying (sodium sulfate), the ether was removed *in vacuo* to yield 2.1 g. (6.8 mmoles, 73%) of a hygroscopic, white solid, m.p. 80–81°; $[\alpha]_D$ −91° (c 0.22%, C_2H_5OH).

Anal.—Calc. for $C_{12}H_8F_5NO_3$: C, 46.60; H, 2.58; N, 4.53. Found: C, 46.39; H, 2.52; N, 4.68.

***N*-Pentafluorobenzoyl-S-(−)-prolyl 1-Imidazolide (I)**—A solution of *N,N'*-carbonyldiimidazole (1.47 g., 9.07 mmoles) in 20 ml. anhydrous tetrahydrofuran was added over a 15-min. period with stirring to an ice-cold solution of Compound III (2.94 g., 9.51 mmoles) in 50 ml. anhydrous tetrahydrofuran. Stirring was continued for an additional 30 min., after which the white crystalline solid that had separated during the reaction was collected to yield 2.2. g. (6.13 mmoles, 64.4%) of the analytically pure imidazolide, m.p. 175–178°; $[\alpha]_D$ −34° (c 0.10%, $CH_3CO_2C_2H_5$).

Anal.—Calc. for $C_{15}H_{10}F_5NO_2$: C, 50.14; H, 2.80; N, 11.69. Found: C, 50.20; H, 2.90; N, 11.63.

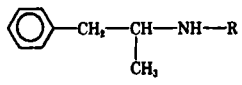
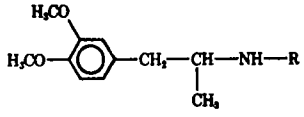
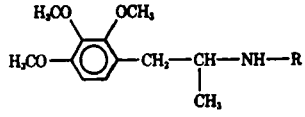
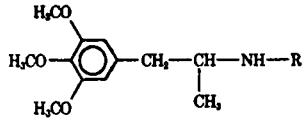
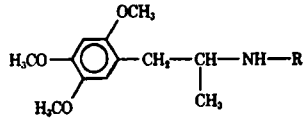
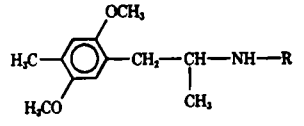
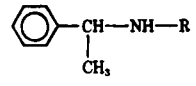
(−)-*N*¹-Pentafluorobenzoyl-*N*²-S-β-phenylisopropyl-S-prolylamide (IV*b*)—A solution of S-(+)-β-phenylisopropylamine (187 mg., 1.39 mmoles) in 10 ml. dry benzene was added to a suspension of Compound I (500 mg., 1.39 mmoles) in 2 ml. dry tetrahydrofuran. One hour later, the reaction mixture was shaken with 2 ml. water. The benzene layer was separated, dried (sodium sulfate), and concentrated *in vacuo*. The resulting solid was crystallized from 50% aqueous methanol to yield 500 mg. (1.17 mmoles, 84.7%) of amide IV*b*, m.p. 70–74°; $[\alpha]_D$ −84° (c 0.2%, C_2H_5OH).

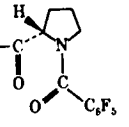
Anal.—Calc. for $C_{21}H_{19}F_5N_2O_2$: C, 59.11; H, 4.49; N, 6.57. Found: C, 59.29; H, 4.62; N, 6.39.

Apparatus—The GLC work was performed on a chromatograph[1] equipped with a ^{63}Ni detector. A 2.12-m. (6-ft.) × 0.31-cm. (0.125-in.) diameter glass column, packed with 3% OV-17 coated onto Chromo-

[1] Varian 1200.

Table I—Gas–Liquid Partition Chromatography[a] Retention Times of *N*-Pentafluorobenzoyl-S-prolyl Derivatives of Various Amines

Compound		Retention Time, min. Derived from			
Number	Structure[b]	Levorotatory Amine *a*	Dextrorotatory Amine *b*	Column Temperature	Resolution Factor[c]
IV		11.25	12.00	250°	0.6
V		12.48	14.40	250°	1.1
VI		16.50	17.75	270°	1.0
VII		13.00	15.00	270°	1.2
VIII		11.8	13.2	270°	1.2
IX		11.4	12.7	270°	1.1
X		10.5	9.0	245°	1.2

[a] Nitrogen flow, 25 ml./min.; injection temperature, 270°; and detector temperature, 300°. [b] R =  [c] See *Reference 14*. Note that when the resolution factor = 1, the peak resolution is approximately 98% complete.

sorb W AWDMCS HP, 100–120 mesh, was used. The column was conditioned for 48 hr. at 280°, the first 24 hr. without nitrogen flow.

Procedure for GLC Analyses—The amine (0.01–0.02 mmole) in anhydrous benzene (1 ml.) was treated with an equimolar amount of Compound I dissolved in 1 ml. anhydrous benzene for 30 min. at room temperature. The reaction mixture then was shaken with 1 ml. water for 30 min. and centrifuged at 2000 r.p.m. for 1 min. The benzene solution (1–5 μl.) was then injected onto the column.

Measurement of Electron-Capture Response—Amide IV*b*, or the diastereomeric mixture obtained with racemic amphetamine and Compound I, was injected onto the column in an amount giving a response that did not exceed 30% of the standard potential (11). The area under the peak was calculated as the product of peak height and width at half the peak height. By knowing the current for full-scale deflection at the sensitivity setting of the amplifier and the chart speed used, the area measurements were converted to coulombs. Response was then expressed as coulombs per mole injected.

RESULTS AND DISCUSSION

It was previously shown (11, 13) that the enhanced electron-capture sensitivity of the pentafluorobenzoylamides of primary and secondary amines over the corresponding trifluoroacetyl derivatives is a consequence of the greater resonance stabilization of the captured electron by the pentafluorobenzoyl moiety. Consequently, it was anticipated that pentafluorobenzoylprolyl derivatives would display electron-capture sensitivity. Quantitative estimations of the amide IV*b*, derived from S-(+)-amphetamine and Compound I, established the electron-capture sensitivity to be 2.4×10^3 coulombs/mole, comparable to the reported sensitivity of related derivatized secondary amines (11). The diastereomeric mixture consisting of amides IV*a* and IV*b*, obtained with racemic β-phenylisopropylamine and Compound I, gave two well-resolved peaks (Table I) of equal intensity on gas–liquid partition chromatographic analysis. When utilizing this derivatizing agent, as little as 1 ng. of these diastereomeric amides can be simultaneously detected. Thus, a sensitive method to estimate enantiomeric composition as well as the potential for quantitative estimation of these amines from biological fluids is established.

To investigate further the potential value of this reagent, the amines listed in Table I that were available either fully or partially resolved were converted to their corresponding *N*-pentafluorobenzoylprolyl derivatives and analyzed by gas–liquid partition chromatography. In the case of the six β-phenylisopropylamines (IV–IX, R = H), it was observed that the retention times of the levorotatory compounds were consistently shorter than the retention times of the corresponding dextrorotatory compounds. The absolute configurations of amphetamine (IV) (15), 1-(3,4-dimethoxyphenyl)-2-aminopropane (V) (16), 1-(3,4,5-trimethoxyphenyl)-2-

aminopropane (VII)[2], and 1-(2,5-dimethoxy-4-methyl)-2-aminopropane (IX)[2] were all established as R(−) and S(+). Consequently, it is tempting to assume a correlation between retention time and absolute configuration. Consistent with such an assumption is the observation that the amide of R-(+)-α-methylbenzylamine (X*b*) has a shorter retention time than the amide of S-(−)-α-methylbenzylamine (X*a*). However, until more information on the molecular nature of the interactions of such diastereomers with column materials is available, this suggestion must remain speculative. Similar differences in retention times with configuration of a series of camphorsulfonamides (17) of α-methoxy-α-methylpentafluorophenylacetamides (18) and of *N*-trifluoroacetylprolylamides (19) have been reported.

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[2] The absolute configurations of these compounds were established by optical rotatory dispersion and circular dichroism analyses of the resolved amines (*via o*-nitrotartranilate salts). The details of these studies will be submitted for future publication.

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▲ To whom inquiries should be directed.

Degradation of Bronchodilator Agents in Oxymix System

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Abstract □ Known amounts of isoproterenol, phenylephrine, and epinephrine were added to the aqueous oxymix system and thereby exposed to potential redox destruction. Thirty minutes later, recoveries of isoproterenol and phenylephrine were 89–100% and 89–108%, respectively, while the recovery of epinephrine was 53–79%.

Keyphrases □ Bronchodilator agents—degradation in oxymix system, clinical implications of concurrent use □ Oxymix–isoproterenol, phenylephrine, or epinephrine—degradation of bronchodilators, clinical implications of concurrent use □ Fluorometry—determination of stability of isoproterenol, phenylephrine, and epinephrine in aqueous oxymix system

Oxymix[1] is a mixture of ascorbic acid, cupric sulfate, sodium percarbonate, a buffer system, and excipients and is essentially a redox system. It has mucolytic properties and has been clinically administered as an aerosol in the treatment of several pathologic pulmonary conditions (1). Because bronchodilating agents are often used for the same indications, knowledge of the compatibility of representative bronchodilators and oxymix was considered necessary, particularly since concurrent administration might be preferred. Since no investigations of the stability of catecholamines or catecholamine-like compounds under such conditions had been reported previously, the stability of epinephrine, isoproterenol, and phenylephrine in the oxymix system was determined using fluorometric assay methods.

[1] Ascoxal (Gum-ox, Ascumist), marketed for oral hygiene by Astra Läkemedel, Södertälje, Sweden.

EXPERIMENTAL

Apparatus—The fluorometric measurements were performed with two types of fluorometers[2]. The ion-exchange columns were of the same design and dimensions as described by Kelly and Auerbach (2) but contained 50 × 5-mm. resin beds.

[2] G. K. Turner Associates, model 111: primary filter No. 110-812 (405 nm.) and secondary filter No. 110-825 (65A) (495 nm.). Aminco-Bowman, model 4-8202: excitation 270 nm., emission 305 nm., slit arrangement 5, xenon lamp 416-992, photomultiplier 10-213, meter multiplier 0.001, and sensitivity setting 46.