

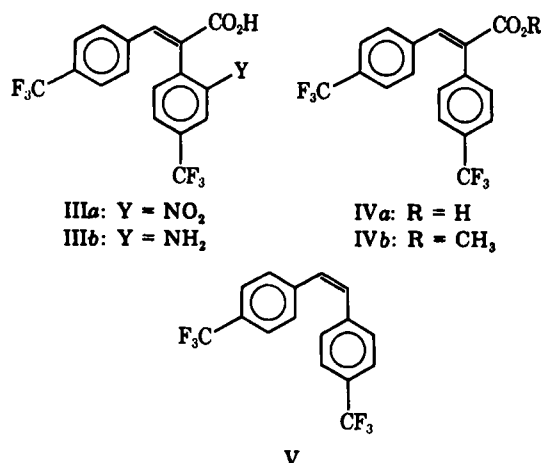
Synthesis and Antimalarial Activity of Benzotrifluoride Derivatives

JAROSLAV NOVOTNY, CAROL H. COLLINS, and FRED W. STARKS[▲]

Abstract □ The preparation of the potential antimalarial agents 4,4'-bis(trifluoromethyl)stilbene, 4,4'-bis(trifluoromethyl)- α -phenylcinnamic acid, and several trifluoromethyl-bearing intermediates is described. Use of a modified Willgerodt synthesis provided the best synthetic route to 4-trifluoromethylphenylacetic acid. A modification of the Grignard synthesis of 4-trifluoromethylbenzaldehyde and 1-(4-trifluoromethylphenyl)ethanol, suitable for largescale preparation, is reported.

Keyphrases □ Benzotrifluoride derivatives—synthesized as potential antimalarials □ 4,4'-Bis(trifluoromethyl)stilbene—synthesized as potential antimalarial □ 4,4'-Bis(trifluoromethyl)- α -phenylcinnamic acid—synthesized as potential antimalarial □ Antimalarial agents, potential—synthesis of benzotrifluoride derivatives

The antimalarial activity of a number of 3- and/or 6-trifluoromethyl-substituted phenanthrenemethanol analogs (1–4) has prompted extensive studies of one of the most promising, α -(2-piperidyl)-3,6-bis(trifluoromethyl)-9-phenanthrenemethanol hydrochloride (1, 3, 5). Practical largescale preparation of this and related compounds requires use of inexpensive starting materials in synthetic routes amenable to scale-up. This article reports the preparation of several benzotrifluoride derivatives useful for the preparation of the related phenanthrenes. The reaction sequences described represent new approaches to the preparation of these compounds. Of particular interest is a new preparation of the Grignard of 4-chlorobenzotrifluoride suitable to the largescale synthesis of 4-trifluoromethylbenzaldehyde (I) and 1-(4-trifluoromethylphenyl)ethanol (II). The aldehyde (I) is used in the preparation of such important antimalarial intermediates as 4,4'-bis(trifluoromethyl)-2'-nitro- α -phenylcinnamic acid (III) (1), 4,4'-bis(trifluoromethyl)- α -phenylcinnamic acid (IV) (both prepared by the Perkin condensation of I with the appropriate phenylacetic acid), and 4,4'-bis(trifluoromethyl)stilbene (V), prepared using



the Wittig reaction of I with 4-trifluoromethylbenzyltriphenylphosphonium chloride (VI). Compound IIIb is converted to a phenanthrene derivative by the Pschorr process (1), while IVb and V have been photocyclized to the corresponding phenanthrenes¹ (4) for subsequent conversion to 3,6-bis(trifluoromethyl)-9-phenanthrene carbinolamines (6). In addition to its use in the sequence herein, the ethanol (II) is a starting material for the preparation of 2,6-bis(4-trifluoromethylphenyl)-4-pyridinemethanol derivatives, another important family of antimalarial compounds (7).

SYNTHESES

Two routes were investigated for the preparation of the intermediate 4-trifluoromethylphenylacetic acid (VII) from 4-chlorobenzotrifluoride. In one (Route B), 4-chlorobenzotrifluoride was converted to the magnesium Grignard and then to 4-trifluoromethylbenzaldehyde (I) (8) in 43% yield. This was transformed into 4-trifluoromethylbenzyl alcohol (VIII) (9) in 95% yield and then to 4-trifluoromethylbenzyl chloride (IX) (9, 10) in 98% yield. The chloride (IX) was converted to 4-trifluoromethylbenzoxonitrile (X) (11) (66% yield). Hydrolysis of X gave the acid (VII) in 72% yield. The overall yield by this route is 19%.

4-Chlorobenzotrifluoride was chosen as initial starting material due to its lower cost and because of a hazard associated with the use of 3- (and possibly 4-) bromobenzotrifluoride (12). Preparation of I was facilitated by use of an entrainment procedure (13) in tetrahydrofuran (14) to prepare the intermediate Grignard reagent². As a result of this modification, the yield of Grignard reagent has improved from 9% (14) to more than 75%. Preparation of the Grignard and its subsequent reaction with appropriate aldehydes have been successfully carried out in these laboratories up to the 50-mole scale.

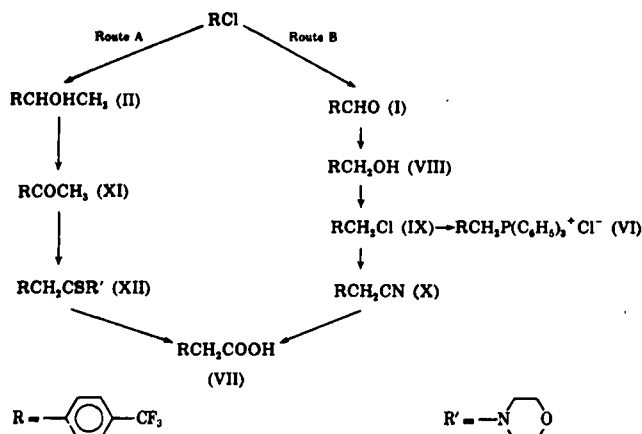
In the conversion of IX to the nitrile (X), a 10% yield of α,β -bis(4-trifluoromethylphenyl)propionitrile (XIII) was formed as a side-product. In an attempt to prepare VIII directly from 4-chlorobenzotrifluoride, the Grignard was reacted with formaldehyde. This produced a 19% yield of the desired VIII and 35% of bis(4-trifluoromethylbenzyl)formal (XIV).

In the second (and preferred) route to 4-trifluoromethylphenylacetic acid (VII) (Route A, Scheme I), the Grignard of 4-chlorobenzotrifluoride was reacted with acetaldehyde to give 1-(4-trifluoromethylphenyl)ethanol (II) (15) (74% yield), which was converted to 4-trifluoromethylacetophenone (XI) (15, 16) by dichromate oxidation in 83% yield. The Willgerodt reaction on XI gave a 72% yield of 4-trifluoromethylphenylthioacetomorpholide (XII). This was hydrolyzed to the acid (VII) in 80% yield. The higher overall yield (35%), the convenience of scale-up to 10 or 20 moles or more, and the lack of complicating side reactions make this Route A the preferred route to VII.

The condensation of 4-trifluoromethylbenzaldehyde (I) with 4-trifluoromethylphenylacetic acid (VII) gave 4,4'-bis(trifluoromethyl)- α -phenylcinnamic acid (IVa) in 94% yield. This was converted to its methyl ester (IVb) by treatment with methanol over molecular sieves.

¹ Largescale photocyclization of both IVb and V was carried out by Dr. R. Srinivasan, IBM Corp. (personal communication).

² The preparation of 4-trifluoromethylbenzylmagnesium chloride was developed by Dr. C. E. Maxwell, Pfizer, Inc., and was communicated to the authors by Dr. Barry M. Bloom of that company.



Scheme I

The 4,4'-bis(trifluoromethyl)stilbene (V) was obtained both from 4-trifluoromethylbenzaldehyde (I) and 4-trifluoromethylbenzyltriphenylphosphonium chloride (VI) and by decarboxylation of IVa. Both methods produce mixtures of the *cis*- and *trans*-isomers which could be separated by fractional distillation. Both isomers (and their mixture) give the same product, 4,4'-bis(trifluoromethyl)-bibenzyl (XV), on hydrogenation.

BIOLOGICAL

The benzotrifluoride derivatives described were tested for anti-malarial activity against *Plasmodium berghei*-infected mice by the test of Osden *et al.* (17). None of these compounds showed antimalarial activity¹.

EXPERIMENTAL⁴

4-(Trifluoromethyl)benzaldehyde (I)—To 121.5 g. (4.998 g.-atoms) of magnesium turnings and 100 ml. of dry tetrahydrofuran was added 14.6 g. (0.134 mole) of ethyl bromide. After the reaction subsided, the mixture was augmented with 1775 ml. of tetrahydrofuran. With stirring, a solution of 257.9 g. (2.367 moles) of ethyl bromide and 457.5 g. (2.534 moles) of 4-chlorobenzotrifluoride⁵ was added dropwise at a rate that maintained a gentle reflux; about 3 hr. was required². The reaction mixture was refluxed for 30 min. and then cooled to 30–35°. While temperature was maintained, 365.0 g. (4.994 moles) of dimethylformamide was added dropwise. After being stirred at 25–30° for 2 hr. the reaction mixture was poured onto ice containing 238 ml. of concentrated sulfuric acid. The tetrahydrofuran was distilled off, and the product was recovered from the aqueous residue by hexane extractions. Crude material was distilled *in vacuo*, yielding 188.8 g. (43%) b.p. 68–70°/13 mm. Hg [lit. (8) b.p. 66–67°/13 mm. Hg]. The product was homogeneous to VPC; λ_{\max} ($\epsilon \times 10^{-3}$): 236 (11.4), 280 (1.3), and 287 (1.2) nm.

Anal.—Calc. for $C_8H_5F_3O$: C, 55.18; H, 2.90; F, 32.73. Found: C, 54.96; H, 2.82; F, 32.34.

4-(Trifluoromethyl)benzyl Alcohol (VIII)—A solution of 87 g. (0.50 mole) of I in 100 ml. of ether was added dropwise to 7.6 g. (0.20 mole) of lithium aluminum hydride in 200 ml. of ether. The suspension was refluxed for 1 hr., cooled, hydrolyzed by the addition of 100 ml. of water, and then filtered. The ether was removed from the filtrate by spin-evaporation to produce an oily residue of

the benzyl alcohol suitable for further transformation, yielding 83.5 g. (95%). Analytically pure material was obtained by distillation *in vacuo*; b.p. 97°/21 mm. Hg [lit. (9) b.p. 86°/8 mm. Hg]. The product was homogeneous to VPC and TLC; λ_{\max} ($\epsilon \times 10^{-3}$): 257 (3.2), 262 (3.3), and 268 (2.5) nm.

Anal.—Calc. for $C_8H_7F_3O$: C, 54.55; H, 4.01; F, 32.36. Found: C, 54.49; H, 4.10; F, 32.16.

4-(Trifluoromethyl)benzyl Chloride (IX)—To a solution of 17.6 g. (0.100 mole) of crude VIII and 7.9 g. (0.10 mole) of pyridine in 100 ml. of chloroform was added dropwise 15.7 g. (0.132 mole) of thionyl chloride. The reaction mixture was refluxed overnight, cooled, washed with water (3400 ml.), dried, and then filtered. The filtrate was dried and then spin-evaporated to an oil, yielding 19.0 g. (98%). The product was suitable for further transformation. Analytically pure material was obtained by distillation *in vacuo*; b.p. 67°/10 mm. Hg [lit. (9, 10) b.p. 61°/8 mm. Hg]. This was homogeneous to both VPC and TLC; λ_{\max} ($\epsilon \times 10^{-3}$): 264 (5.0) and 271 (4.0) nm.

Anal.—Calc. for $C_8H_6ClF_3$: C, 49.38; H, 3.11; Cl, 18.22; F, 29.29. Found: C, 49.47; H, 3.25; Cl, 17.99; F, 29.42.

4-(Trifluoromethyl)phenylacetonitrile (X)—To a stirred suspension of 11.7 g. (0.239 mole) of sodium cyanide in 55 ml. of dimethylformamide at 50° was added dropwise 359 g. (0.181 mole) of IX. Heating was continued for 1.5 hr. The reaction mixture was cooled and diluted with 500 ml. of water, and two layers resulted. This mixture was extracted into ether. The ether extracts were dried and the ether was removed to yield a crude product, which was purified by distillation to yield 22 g. (66%) of X; b.p. 117–126°/14 mm. Hg [lit. (11) b.p. 110–112°/14 mm. Hg]. The compound crystallized on standing; m.p. 43–45° [lit. (11) m.p. 44–45°]. It was homogeneous to TLC; λ_{\max} ($\epsilon \times 10^{-3}$): 256 (3.2), 262 (3.8), and 267 (3.2) nm.

Anal.—Calc. for $C_8H_6F_3N$: C, 58.39; H, 3.26; F, 30.78; N, 7.56. Found: C, 58.13; H, 3.12; F, 30.50; N, 7.49.

1-(4-Trifluoromethylphenyl)ethanol (II)—A solution of 201 g. (4.56 moles) of acetaldehyde in 100 ml. of dry tetrahydrofuran was added during 90 min. to a Grignard reagent prepared from 97.2 g. (4.00 moles) of 4-chlorobenzotrifluoride as described for I. The crude II was isolated in a similar fashion and distilled *in vacuo* to yield 296 g. (74%) of product suitable for further transformation; b.p. 91–103°/10 mm. Hg [lit. (15) b.p. 106–107°/18 mm. Hg].

4-(Trifluoromethyl)acetophenone (XI)—A solution of 426 g. (2.24 moles) of II in 610 ml. of glacial acetic acid was added in six portions (at 10°) to 305 g. (1.04 moles) of sodium dichromate in 460 ml. of glacial acetic acid. The reaction mixture was heated at 80° for 1 hr., cooled, added to 15 l. of cold water, and then extracted into ether (8 × 5 l.). The ether was washed with saturated aqueous sodium carbonate (1500 ml.) and water (2 × 3 l.), dried, and then filtered. The filtrate was spin-evaporated to a residue. The product was distilled to yield 330 g. (83%) b.p. 81–83°/10 mm. Hg [lit. (14, 15) b.p. 79–80°/8 mm. Hg] and further purified by recrystallization from 170 ml. of petroleum ether (b.p. 30–60°); m.p. 30–31°. The product was homogeneous to VPC and TLC; λ_{\max} ($\epsilon \times 10^{-3}$): 233 (13.1) and 278 (1.4) nm.

Anal.—Calc. for $C_8H_7F_3O$: C, 57.45; H, 3.75; F, 30.29. Found: C, 57.64; H, 3.76; F, 30.20.

4-(Trifluoromethyl)phenylthioacetomorpholide (XII)—A mixture of 2171 g. (11.50 moles) of XI, 1503 g. (17.25 moles) of morpholine, and 550 g. (17.3 g.-atoms) of sulfur was refluxed for 16 hr., cooled, and then poured into 2300 ml. of ethanol containing 45 ml. of benzene. The crystalline product which precipitated was suitable for further transformation, yielding 2396 g. (72%). Analytically pure material was obtained by recrystallization from ethanol, m.p. 120–120.5°. The compound was homogeneous to TLC (four solvent systems); λ_{\max} ($\epsilon \times 10^{-3}$): 282 (14.4) nm.

Anal.—Calc. for $C_{13}H_{14}F_3NOS$: C, 53.97; H, 4.88; N, 4.84. Found: C, 53.88; H, 4.92; N, 4.86.

4-(Trifluoromethyl)phenylacetic Acid (VII)—*Method A*—A solution of 2396 g. (8.030 moles) of XII in 32.4 l. of 10% aqueous potassium hydroxide was refluxed for 16 hr. The mixture was filtered, and the filtrate was added slowly to a cold (5°) solution of 7.8 l. of concentrated hydrochloric acid in 32 l. of water. The resulting crystalline precipitate was collected and recrystallized from petroleum ether (b.p. 30–60°), yielding 1300 g. (80%), m.p. 81–83° [lit. (11) m.p. not recorded]. The product was homogeneous to TLC; λ_{\max} ($\epsilon \times 10^{-3}$): 257 (2.8), 202 (3.0), and 268 (2.3) nm.

Anal.—Calc. for $C_8H_7F_3O_2$: C, 52.95; H, 3.46; F, 27.92. Found: C, 53.14; H, 3.45; F, 28.00.

¹ The test results were provided by the Walter Reed Army Institute of Research through the courtesy of Dr. T. R. Sweeney and Dr. B. T. Poon.

⁴ All temperatures reported are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. VPC was carried out using a Hewlett-Packard model 5750B, temperature programmed at 10°/min. from 40 to 250°, using a 2-m. × 3-mm. column packed with 10% UC-W982 on 80–100-mesh Diataport S. TLC samples were developed on Eastman Chromagram Sheet 6060 using at least three solvent systems for each compound. UV spectra were measured in ethanol on a Bausch & Lomb Spectronic 505 spectrophotometer.

⁵ Hooker Chemical Co.

Method B—A mixture of 10 ml. concentrated sulfuric acid, 10 ml. water, 15 ml. glacial acetic acid, and 10 g. (0.05 mole) of X was refluxed for 2 hr. and then cooled. Two layers formed which were saturated with ether (4 × 50 ml.). The ether extracts were dried and spin-evaporated to yield an oil which crystallized on standing. Recrystallization yielded 6.9 g. (72%) of VII, m.p. 81–83°, identical to that obtained above.

4,4'-Bis(trifluoromethyl)- α -phenylcinnamic Acid (IVa)—To a suspension of sodium methoxide (11.6 g., 0.215 mole) in 1000 ml. of acetic anhydride was added, under a nitrogen atmosphere, 43.9 g. (0.215 mole) of VII. The solution was stirred 20 min., 25 g. (0.14 mole) of I was added, and the mixture was stirred at 100° for 18 hr. While still at 100°, four portions of water were added to hydrolyze the acetic anhydride. During this process a crystalline product appeared which was collected after cooling the mixture to 20°, yielding 48.8 g. (94%), m.p. about 140°. This material contained mostly *cis*-IVa with some *trans*-IVa and was suitable for further transformation. Pure *cis*-IVa was obtained by chromatographing the mixture on silica gel with a 7:3 mixture of petroleum ether (b.p. 30–60°) and ethyl acetate, m.p. 184–185° (lit.⁶ m.p. 183–186°). This material was homogeneous to TLC; λ_{\max} ($\epsilon \times 10^{-4}$): 275 (1.4) nm.

Anal.—Calc. for $C_{17}H_{10}F_6O_2$: C, 56.68; H, 2.80; F, 31.64. Found: C, 56.62; H, 2.78; F, 31.73.

Methyl 4,4'-Bis(trifluoromethyl)- α -cinnamate (IVb)—A flask containing 48.8 g. (0.135 mole) of crude IVa in 250 ml. of methanol and 25 ml. of concentrated sulfuric acid was attached to an apparatus (soxhlet) containing 85 g. of No. 3A molecular sieves immersed in 1000 ml. of methanol. After refluxing for 40 hr., the reaction mixture was poured into 1000 ml. of cold water containing 66 g. of K_2CO_3 . The mixture was extracted with ether. The ether extracts were washed with water, dried, and spin-evaporated to a residue which was chromatographed on 100 g. basic alumina with ether as the eluate. After removing the ether, the resulting oil crystallized on standing, yielding 47 g. (93%). This was further purified by distillation *in vacuo*, b.p. 127–133°/0.6 mm. Hg. VPC and TLC revealed the presence of two isomers, with *cis*-IVb predominating (VPC); λ_{\max} ($\epsilon \times 10^{-4}$): 279 (1.65) nm.

Anal.—Calc. for $C_{18}H_{12}F_6O_2$: C, 57.76; H, 3.23; F, 30.46. Found: C, 57.80; H, 3.40; F, 30.21.

Pure *cis*-IVb was obtained by fractional distillation *in vacuo*, b.p. 141–143°/1.5 mm. Hg, followed by crystallization from hexane, m.p. 70–71° (lit.⁶ m.p. 69–72°). *cis*-IVb was homogeneous to VPC and TLC; λ_{\max} ($\epsilon \times 10^{-4}$): 277 (2.0) nm.

Anal.—Calc. for $C_{18}H_{12}F_6O_2$: C, 57.76; H, 3.23; F, 30.46. Found: C, 57.99; H, 3.06; F, 30.28.

4-(Trifluoromethyl)benzyltriphenylphosphonium Chloride (VI)—A mixture of 8.3 g. (43 mmoles) of IX and 11.8 g. (44.8 mmoles) of triphenylphosphine in 200 ml. of dry xylene was refluxed overnight and then cooled. The crystalline product which separated was filtered off and dried, yielding 17.3 g. (88%), m.p. 275–281° dec. Analytically pure material was obtained by a single recrystallization from ethanol, m.p. 283–285° dec. It was homogeneous to TLC; λ_{\max} ($\epsilon \times 10^{-3}$): 267 (3.7) and 274 (2.9) nm.

Anal.—Calc. for $C_{26}H_{21}ClF_3P$: C, 68.35; H, 4.63; F, 12.48; P, 6.78. Found: C, 68.13; H, 4.74; F, 12.66; P, 6.79.

4,4'-Bis(trifluoromethyl)stilbene (V)—**Method A**—To a suspension of 21.5 g. (47.1 mmoles) of VI under nitrogen was added 36.7 g. of hexane solution containing 61 mmoles of butyllithium. The reaction mixture was stirred for 2 hr., and then 9.9 g. (57 mmoles) of I was added. The suspension was stirred for 16 hr., diluted with petroleum ether (b.p. 30–60°) (1175 ml.), filtered, and then spin-evaporated to an oil. Addition of 500 ml. of petroleum ether (b.p. 30–60°) precipitated the triphenylphosphonium oxide, which was discarded. The filtrate was spin-evaporated *in vacuo*, and the resulting residue was distilled to give 4.8 g. (32%) of *cis*-V, b.p. 80–82°/2 mm. Hg, m.p. 49–52°. *trans*-V, 7.4 g. (50%), was recovered from the residue, m.p. 124–127°. Assignment of configuration was by comparison of physical properties and spectra with those of authentic samples of the unsubstituted stilbene isomers (18–20). Each isomer was homogeneous to VPC (retention time 14.2 and 16.7 min. for *cis*- and *trans*-V, respectively); λ_{\max} (*cis*) ($\epsilon \times 10^{-4}$): 225 (2.1) and 280 (1.3) nm.; λ_{\max} (*trans*) ($\epsilon \times 10^{-4}$): 226 (1.4), 298 (3.1), 308 (3.2), and 320 (2.0) nm.

Anal.—Calc. for $C_{16}H_{10}F_4$: C, 60.79; H, 3.19; F, 36.04. Found for *cis*-V: C, 60.80; H, 3.27; F, 35.85. Found for *trans*-V: C, 60.78; H, 3.14; F, 36.20.

Method B—A stirred mixture of 20.0 g. (0.056 mole) of IVa, 70 ml. of quinoline, and 1 g. of copper powder was refluxed (at 195°) for 17 hr. The reaction mixture was poured into 280 ml. of 10% HCl and extracted into ether. The ether was removed, and the product mixture was chromatographed on alumina using hexane. Distillation under vacuum gave 9.5 g. (54%) of mixed *cis*- and *trans*-V, identified by VPC. Fractional distillation gave 0.7 g. of *cis*-V and 8.5 g. of *trans*-V.

1,2-Bis(4-trifluoromethylphenyl)ethane (XV)—A mixture of *cis*- and *trans*-V (6.4 g., 20 mmoles) in glacial acetic acid was hydrogenated for 30 min. over 400 mg. of palladium oxide. The solution was neutralized with 50% NaOH and extracted with ether. The extracts were dried and spin-evaporated to a crystalline residue. Analytically pure material was obtained by recrystallization from hexane, yielding 5.2 g. (81%), m.p. 78–79°. The product was homogeneous to VPC and TLC; λ_{\max} ($\epsilon \times 10^{-3}$): 253 (5.5), 258 (7.0), 263.5 (5.9), and 268.5 (3.9) nm.

Anal.—Calc. for $C_{16}H_{12}F_8$: C, 60.38; H, 3.80; F, 35.82. Found: C, 60.30; H, 3.90; F, 35.61.

α,β -Bis(4-trifluoromethylphenyl)propionitrile (XIII)—This product was obtained as a by-product of the reaction to produce X from IX. The distillation residue was triturated with petroleum ether (b.p. 30–60°) to give a solid, which was then recrystallized twice from absolute ethanol (charcoal), m.p. 78–79°. The material was homogeneous to TLC. The compound was characterized by its molecular weight and IR and NMR spectra; λ_{\max} ($\epsilon \times 10^{-3}$): 258 (8.4), 263 (9.6), and 269 (7.9) nm.

Anal.—Calc. for $C_{17}H_{11}F_6N$: C, 59.48; H, 3.63; F, 33.21; N, 4.08. Found: C, 59.49 and 59.76; H, 3.11 and 3.16; F, 33.55; N, 3.99.

4,4'-Bis(trifluoromethyl)benzylformal (XIV)—This material was obtained during an attempt to prepare VIII directly from 4-chlorobenzotrifluoride, using paraformaldehyde on the intermediate magnesium Grignard. A 19% yield of the desired product, VIII, was obtained. Further fractionation produced XIV in 35% yield, b.p. 119–121°/0.25 mm. Hg. It was homogeneous to VPC and TLC. The compound was identified by its IR and NMR spectra; λ_{\max} ($\epsilon \times 10^{-3}$): 257 (8.0), 262 (9.0), and 268 (7.3) nm.

Anal.—Calc. for $C_{17}H_{11}F_6O_2$: C, 56.05; H, 3.85; F, 31.29. Found: C, 56.18; H, 4.09; F, 30.99.

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ACKNOWLEDGMENTS AND ADDRESSES

Received June 7, 1972, from *Starks Associates, Inc., Buffalo, NY 14213*

Accepted for publication December 20, 1972.

Supported by the U. S. Army Medical Research and Development Command under Contract DA-49-193-MD-2751. This is Contribution 1081 of the Army Research Program on Malaria.

The authors thank Mr. Walter Schreiner, Mrs. Anita White, and Mr. John Whittleton for their valuable contributions to the synthetic work.

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Fluorometric Estimation of Oxytetracycline in Blood and Plasma

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Abstract □ A sensitive spectrofluorometric method was developed for the estimation of oxytetracycline in whole blood or plasma. The method involves the use of protein precipitation, solvent extraction, and alkaline degradation procedures. Reproducible recoveries of oxytetracycline are obtained by adding β -mercaptopropionic acid at various stages of the extraction procedure. Whereas the alkaline decomposition product of oxytetracycline fluoresces at 337/410 nm., the reproducibility of the assay is improved by scanning the fluorescence spectrum from 300 to 500 nm. This enables any variation in background fluorescence of different samples to be estimated and accounted for when measuring low levels of oxytetracycline. The method will measure oxytetracycline to better than 0.1 mcg./ml. blood or plasma and is therefore suitable for monitoring the blood levels obtained after a therapeutic dose. The results obtained in man illustrate the variability of blood level profiles of oxytetracycline following its oral administration under closely controlled conditions.

Keyphrases □ Oxytetracycline—spectrophotofluorometric analysis in whole blood or plasma □ Plasma levels, oxytetracycline—after oral administration in man, spectrophotofluorometric analysis □ Spectrophotofluorometry—analysis, oxytetracycline in whole blood or plasma

A growing understanding of the importance of the blood levels of drugs and their relevance to the clinical evaluation makes it imperative that relatively simple and reliable methods are available for estimating this class of organic compounds in biological fluids. The present study started as an attempt to show biological equivalence, or a lack of it, in oxytetracycline hydrochloride¹ formulations and to correlate blood levels with disintegration times or dissolution times.

Initial problems occurred in the development of a satisfactory analytical method for oxytetracycline in serum, plasma, or whole blood. Although bioassay techniques have been recommended and widely used (1), an alternative method was preferred. Estimations

by conventional polarography offered a rapid, although insensitive, method of analysis, and this could not be improved sufficiently even by the use of polarography (a.c.)⁽²⁾. The more recent colorimetric assays for tetracyclines (3) also lack sensitivity. A variety of fluorescence methods have been reported in the literature; Hayes and DuBuy (4) utilized the conversion to the anhydro-derivative on heating in acid, followed by a measurement of the fluorescence intensity at alkaline pH. Ibsen *et al.* (5) developed a method based on the increase in fluorescence of the oxytetracycline on chelation with magnesium, and Kahn (6) described its estimation in biological systems utilizing the extractability of fluorescent calcium-barbital complexes.

In this laboratory the reported fluorescence methods were largely unworkable and at best gave irreproducible results, probably due to photodecomposition of the oxytetracycline. During the investigations it was found that the ready decomposition which occurred during the various isolation stages could be most satisfactorily prevented by the inclusion of thiol reagents during the work-up procedures. This led to the development of a reliable, sensitive fluorometric method for the quantitation of oxytetracycline at the therapeutic levels encountered in whole blood or plasma.

EXPERIMENTAL³

Reagents—All hydrochloric acid solutions were prepared from concentrated hydrochloric acid³ for Foodstuffs Analysis grade. This was free from the interfering materials often added to some commercially standardized solutions to prevent bacterial growth.

The following aqueous solutions were prepared in glass-distilled water: 0.1 N HCl containing β -mercaptopropionic acid (1.0 mg./ml.), trichloroacetic acid (30% w/v), 1.0 N NaOH, 0.02 N HCl, and

² All fluorometric measurements were carried out using either the Aminco-Bowman spectrophotofluorometer or the Hitachi MPF 2A or MPF 3 instruments. All glassware was cleaned preferably in Haemosol, rinsed in tap water, soaked overnight in 1 N HCl, and, after rinsing in distilled water, dried.

³ British Drug Houses.

¹ Oxytetracycline hydrochloride is the active drug substance in the pharmaceutical formulation Imperacin, marketed by I.C.I. Ltd., Pharmaceuticals Division.