pentylamino]quinoline *d*-Tartrate (V)—This compound was prepared from salicylaldehyde by the procedure previously described. A yield of 96% was obtained, mp 90–95° (with bubbling at 80°).

4-Phthalimidomethylbenzyl Chloride (XII)—1,4-Bis(chloromethyl)benzene (43.7 g, 0.25 mole) was added to 300 ml of dimethylformamide. Potassium phthalimide (46 g, 0.25 mole) was added in two portions. The second portion was added after 3 hr together with another 300 ml of dimethylformamide. The mixture was heated for 3 hr at 85°, and 500 ml of dimethylformamide distilled off under aspirator vacuum (about 18–19 hr). The residue was poured into water, and a white material was collected. This material was extracted with 1 liter of acetone, leaving 18.5 g of the insoluble diphthalimido product. Extraction of the acetone solution with hot petroleum ether (bp 30–60°) removed 12 g of unreacted 1,4-bis(chloromethyl)benzene. From the acetone solution was obtained 33.5 g of desired product, mp 143–146°.

5 - (*p*-Anisyloxy)-6-methoxy-8-(4-phthalimidomethylbenzylamino)quinoline (XIII)—Compound XII (2.8 g), 1.6 g of potassium iodide, and 20 ml of acetone were boiled for 1 hr and concentrated. The aminoquinoline (X) (3 g) was added together with 1.3 g of anhydrous potassium carbonate and 20 ml of 2-propanol. This mixture was heated in an open flask on a steam bath for 1.5 hr, allowing the 2-propanol to evaporate. The residue was poured into water, made basic with potassium carbonate, extracted with chloroform, and dried (sodium sulfate).

The chloroform was removed *in vacuo*, and the residue was heated with absolute ethanol to give an orange solid. This solid was dissolved in benzene, treated with charcoal, filtered, and concentrated to an oily residue. Addition of warm absolute ethanol gave an orange solid, which was collected and washed with absolute ethanol, mp 164–165°. The yield was 3 g (55%). In a second experiment, the yield was increased to 76%.

Anal.—Calc. for $C_{33}H_{27}N_3O_5$: C, 72.65; H, 4.99; N, 7.70. Found: C, 72.82; H, 5.04; N, 7.58.

5-(*p*-Anisyloxy)-6-methoxy-8-(4-aminomethylbenzylamino)-quinoline Maleate (VI)—The phthalimido compound (XIII) (11 g, 0.02 mole) in 200 ml of 95% ethanol and 10 ml of 85% hydrazine hydrate was refluxed for 1.5 hr at 90–100°. Ethanol was removed, 20 g of potassium hydroxide in 20 ml of water was added, and the mixture was stirred for 0.5 hr. The solution was extracted with ether (500 ml), dried over sodium sulfate, concentrated, and redissolved in ether. A saturated ether solution of maleic acid (11 g/500 ml of ether) was added. The yellowish orange precipitate (hygroscopic) was filtered, dissolved in chloroform, and reprecipitated with ether, mp 136–139°. It analyzed as the dimaleate salt.

Anal.—Calc. for $C_{25}H_{25}N_3O_3 \cdot (C_4H_4O_4)_2$: C, 61.20; H, 5.16; N, 6.49. Found: C, 60.91; H, 5.24; N, 6.72.

Two additional reprecipitations from chloroform by ether gave the product as the monomaleate salt (77.5%), mp 158–160° (Table I).

REFERENCES

(1) K. Paul and C. D. Blanton, Jr., *J. Med. Chem.*, **16**, 1391(1973).

(2) W. P. Wetter and C. D. Blanton, Jr., *ibid.*, **17**, 620(1974).

(3) N. L. Drake, R. A. Hayes, J. A. Garman, R. B. Johnston, G. W. Kelley, S. Melamed, and R. M. Peck, *J. Am. Chem. Soc.*, **71**, 455(1949).

(4) G. R. Coatney, W. C. Cooper, N. B. Eddy, and J. Greenberg, "Survey of Antimalarial Agents," Public Health Monograph No. 9, U.S. Government Printing Office, Washington, D.C., 1953, p. 47.

(5) P. B. Russell, in "Medicinal Chemistry," 2nd ed., A. Burger, Ed., Interscience, New York, N.Y., 1960, p. 814.

(6) F. Schonhofer, *Z. Physiol. Chem.*, **274**, 1(1942).

(7) P. E. Thompson and L. M. Werbel, "Antimalarial Agents," Academic, New York, N.Y., 1972, p. 100.

(8) A. Funke, D. Bovet, and G. Montezin, *Ann. Inst. Pasteur, Paris*, **72**, 264(1946).

(9) T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431(1967).

(10) "Chemotherapy of Malaria and Resistance to Antimalarials," *WHO Tech. Rep. Ser.*, No. 529 (1973).

(11) "WHO Report of Procedures for Screening Potential Antimalarial Compounds," in World Health Organization (1972b); WHO/MAL/72.763 (cyclostyled report), WHO, Geneva.

(12) R. C. Elderfield, W. R. Vaughan, B. R. Millward, and J. H. Ross, *J. Org. Chem.*, **23**, 1378(1958).

(13) W. M. Lauer, C. Rondestvedt, R. T. Arnold, N. L. Drake, J. VanHook, and J. Tinker, *J. Am. Chem. Soc.*, **68**, 1546(1946).

ACKNOWLEDGMENTS AND ADDRESSES

Received September 8, 1975, from the *Department of Medicinal Chemistry, School of Pharmacy, University of Georgia, Athens, GA 30602*

Accepted for publication December 1, 1975.

Supported by the U.S. Army Medical and Development Command under Contract DADA 17-71-C1068.

This paper is Contribution No. 1375 from the Army Research Program on Malaria.

The authors thank Dr. R. E. Strube, Dr. E. A. Steck, and Dr. T. R. Sweeney of the Walter Reed Army Institute of Research for interest and encouragement.

[x] To whom inquiries should be directed.

Determination of Salicylates by GLC

JOHN P. TISCHIO

Abstract □ Silylation of salicylic acid by hexamethyldisilazane, *N,O*-bis(trimethylsilyl)acetamide, and *N,N*-bis(trimethylsilyl)trifluoroacetamide was compared using GLC. The completeness of the reaction, the stability of products, and the reproducibility of results with time were investigated. Different reaction vessels were examined for their reliability and application for a routine assay procedure.

Keyphrases □ GLC—analysis, salicylic acid, different silylating reagents and different reaction vessels compared □ Salicylic acid—GLC analysis, different silylating reagents and different reaction vessels compared □ Silylating reagents, various—compared in GLC analysis of salicylic acid □ Keratolytic agents—salicylic acid, GLC analysis

Several methods for the determination of salicylates in physiological fluids were reviewed previously (1). One of these, GLC, is attractive because it affords a sensitive quantitative method for the simultaneous determination of aspirin and salicylic acid. Several papers have reported different GLC procedures (1–5), but the most promising incorporate a silylation process after extraction of the salicylates from the physiological fluid. The silylating reagents that have been utilized include hexamethyldisilazane (2), *N,O*-bis(trimethylsilyl)-acetamide (3), and *N,N*-bis(trimethylsilyl)trifluoroacetamide (4).

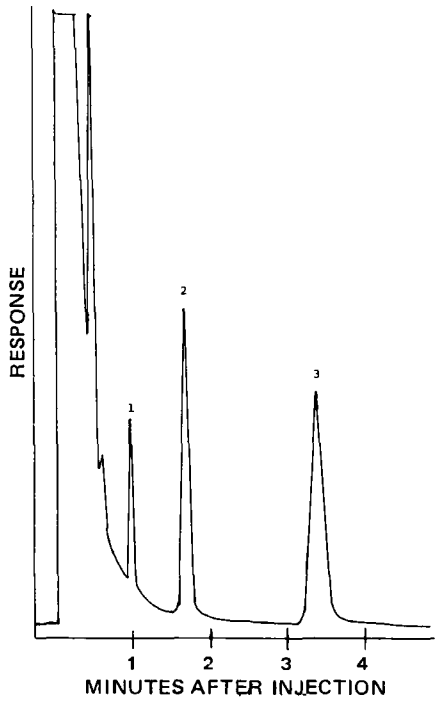


Figure 1—*Typical chromatogram. Key: 1, naphthalene; 2, trimethylsilyl-p-toluic acid; and 3, trimethylsilylsalicylic acid.*

However, when analyzing blood samples for salicylates, different results are obtained depending upon which silylating reagent is used, which reaction vessel is employed, and when the samples are analyzed after derivatization. In this report, a comparison is made of these silylating agents for their completeness of reaction, the stability of trimethylsilylated derivatives in the silylating mixture, and the reproducibility of results. In addition, three different vessels (a polyseal screw-capped centrifuge tube, a ground-glass-stoppered centrifuge tube, and a cork-stoppered centrifuge tube) were examined for their applicability for a routine assay procedure.

EXPERIMENTAL

Materials—The following chemicals were of analytical reagent quality unless otherwise noted: salicylic acid[1] USP, *p*-toluic acid[2], naphthalene[3], acetone[3], anhydrous ether[3], and the silylating reagents hexamethyldisilazane[4], *N,O*-bis(trimethylsilyl)acetamide[4], and *N,N*-bis(trimethylsilyl)trifluoroacetamide[4]. Other materials included screw-capped 15-ml conical centrifuge tubes fitted with polyseal screw caps[5], ground-glass 15-ml conical centrifuge tubes fitted with ground-glass stoppers[5], and plain 15-ml centrifuge tubes fitted with corks[5].

Apparatus—A flame-ionization gas chromatograph[6] was employed with a 1.2-m (4-ft) × 4-mm silanized glass column packed with 3% OV-17 on Gas Chrom Q (100–120 mesh), acid washed and silanized[4].

Analytical Procedure—*GLC Conditions*—The injector and detector temperatures were 200°, the column temperature was 130°, and the helium carrier gas flow rate was 18 ml/min.

Silylation Procedure—A standard stock solution of *p*-toluic acid and salicylic acid was made in anhydrous ether and had a final concentration of 50 μg of each acid/ml. A 0.5-ml aliquot of this solution was pipetted into each of nine silylating vessels (three screw-capped tubes, three ground-glass tubes, and three plain tubes), and each was evaporated on a heating block maintained at 60°. After evaporation, 50 μl of each silylating reagent was added to each type of reaction vessel.

All tubes were flushed with a stream of nitrogen, capped with their respective stoppers immediately, and then incubated in an oven maintained at 60°. After 1 hr, the tubes were removed and allowed to return to room temperature (~5 min). Then 50 μl of an acetone solution containing 100 μg of naphthalene/ml was added to each tube. A 5-μl sample of this last mixture was then subjected to GLC analysis.

[1] Merck and Co.
[2] Matheson, Coleman and Bell Co.
[3] Baker Chemical Co.
[4] Supelco Inc.
[5] Arthur H. Thomas Co.
[6] Gow Mac model 750.

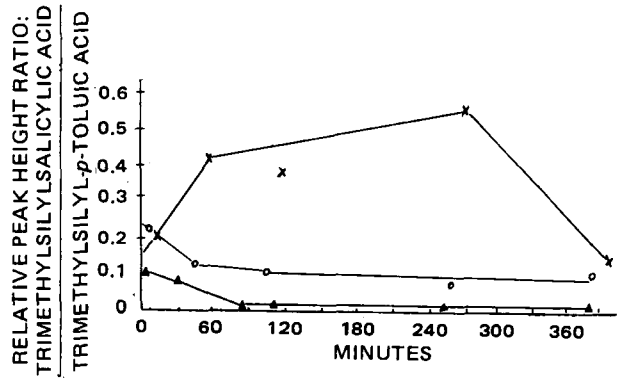


Figure 2—*Comparison of the hexamethyldisilazane reagent in screw-capped tubes (×), ground-glass-stoppered tubes (○), and cork-stoppered tubes (△).*

RESULTS AND DISCUSSION

Hexamethyldisilazane, *N,O*-bis(trimethylsilyl)acetamide, and *N,N*-bis(trimethylsilyl)trifluoroacetamide have been reported as reagents for derivatizing salicylates for their subsequent GLC analysis. This study was conducted over a 6-hr period to determine the relative completeness of the silylation reaction, the stability of trimethylsilyl derivatives in the silylating reagent, and the relative qualitative and quantitative GLC response with respect to time. In addition, several different reaction tubes were investigated as to their suitability for routine analysis.

Figure 1 illustrates a typical *N,N*-bis(trimethylsilyl)trifluoroacetamide chromatogram tracing, showing the separation of naphthalene, trimethylsilyl-*p*-toluic acid, and trimethylsilylsalicylic acid. Their relative retention times under the experimental conditions were 1, 1.8, and 3.5 min, respectively, in close agreement with retention times found by others (4).

Naphthalene, which is not silylatable, is used as an internal standard to ensure consistency in sampling size, with the intensity of its peak not varying more than 3% between samples. The trimethylsilyl-*p*-toluic acid peak is employed as an internal silylatable standard,

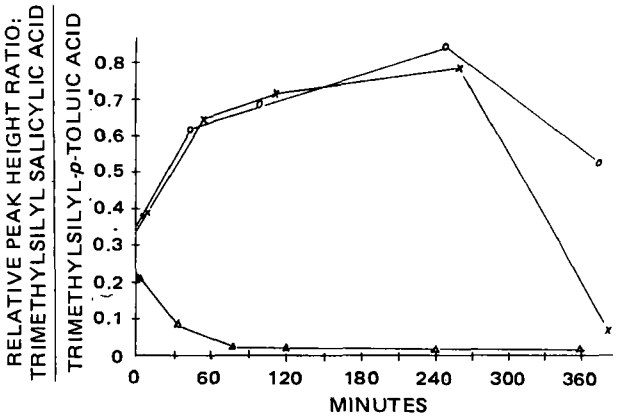


Figure 3—*Comparison of the N,O-bis(trimethylsilyl)acetamide reagent in screw-capped tubes (×), ground-glass-stoppered tubes (○), and cork-stoppered tubes (△).*

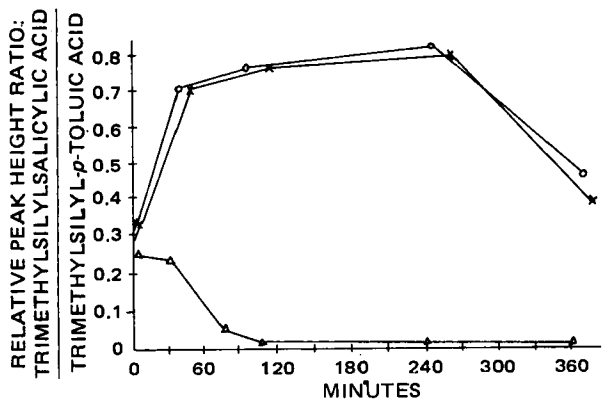


Figure 4—*Comparison of the* N,N-*bis(trimethylsilyl)trifluoroacetamide reagent in screw-capped tubes* (×), *ground-glass-stoppered tubes* (○), *and cork-stoppered tubes* (△).

and the ratio of trimethylsilylsalicylic acid to trimethylsilyl-*p*-toluic acid peaks is a relative measurement of the trimethylsilylsalicylic acid concentration from sample to sample.

The data obtained for each silylating reagent in the three different reaction vessels are compared in Figs. 2–4. In each figure, the ratio of relative peak heights of trimethylsilylsalicylic acid to trimethylsilyl-*p*-toluic acid is plotted *versus* time. All time intervals indicated on the graph for each sample are the times elapsed after removal from incubation.

The hexamethyldisilazane reagent did not silylate the salicylic acid to the same extent as the other reagents under the conditions described. In addition, as time elapsed, the hexamethyldisilazane chromatograms became complex due to the appearance of many interfering peaks (Figs. 5 and 6). Qualitatively as well as quantitatively, this reagent proved to be unsatisfactory.

The *N,O*-bis(trimethylsilyl)acetamide reagent appeared to give the same completeness of derivatization of salicylic acid as *N,N*-bis-(trimethylsilyl)trifluoroacetamide. However, the former showed a deterioration with time as was evident by the peaks tailing and becoming unsymmetrical (Fig. 7).

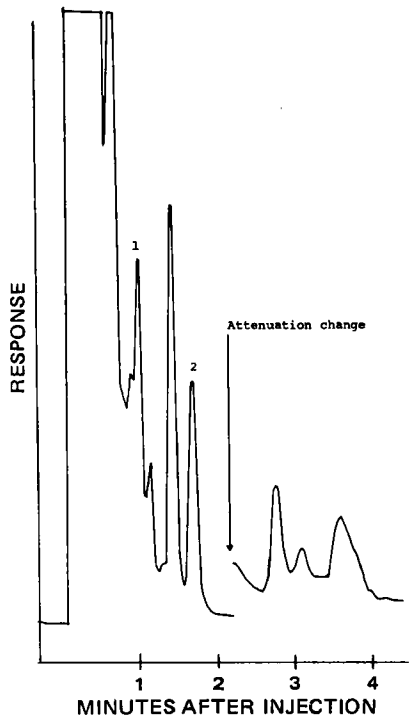


Figure 6—*Typical hexamethyldisilazane gas chromatogram 260 min after derivatization. Key: 1, naphthalene; and 2, trimethylsilyl-*p*-toluic acid.*

The *N,N*-bis(trimethylsilyl)trifluoroacetamide proved to be the best silylating reagent with respect to completeness of derivatization, stability, and reproducibility of the peaks with time under these conditions. Therefore, *N,N*-bis(trimethylsilyl)trifluoroacetamide is the reagent of choice for the silylation of salicylates.

In addition to the differences obtained due to the silylation reagents, the reaction vessel can be a diminishing factor in the amount of measurable derivatives remaining in the tube. Samples silylated in cork-stoppered vessels, whether hexamethyldisilazane, *N,O*-bis-

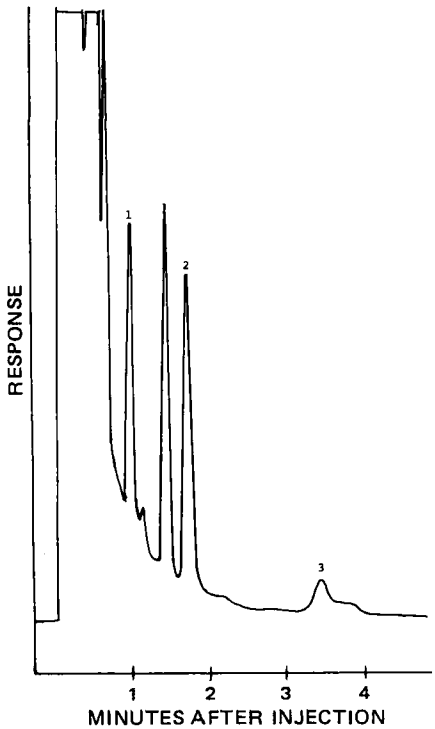


Figure 5—*Typical hexamethyldisilazane gas chromatogram 70 min after derivatization. Key: 1, naphthalene; 2, trimethylsilyl-*p*-toluic acid; and 3, trimethylsilylsalicylic acid.*

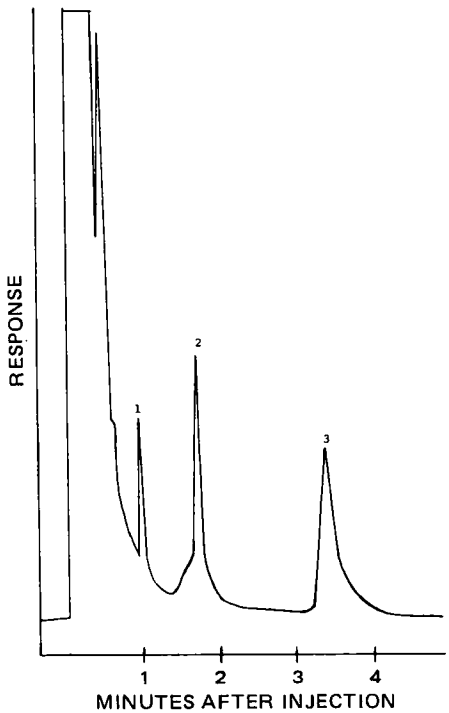


Figure 7—*Typical* N,O-*bis(trimethylsilyl)acetamide gas chromatogram 65 min after derivatization. Key: 1, naphthalene; 2, trimethylsilyl-*p*-toluic acid; and 3, trimethylsilylsalicylic acid.*

(trimethylsilyl)acetamide, or *N,N*-bis(trimethylsilyl)trifluoroacetamide, were totally unsatisfactory. The disappearance of trimethylsilylsalicylic acid with respect to trimethylsilyl-*p*-toluic acid over time was most demonstrable under the cork-stoppered vessel conditions. Polyseal screw-capped and ground-glass-stoppered vessels were satisfactory.

To determine the difficulty with the cork-stoppered tubes, pieces of cork were suspended in three screw-capped tubes, each containing a sample to be silylated along with one silylating reagent. A polyseal cap was used to seal off the tube, and incubation and analysis were performed as described. The chromatograms showed the same pattern as was found for the cork-stoppered vessels for each reagent. The cork probably absorbed the trimethylsilyl derivatives and thereby reduced the amount of measurable derivative.

Regarding the optimal time for injection of samples, the most reliable time for consistent results for salicylic acid was between 1 and 4 hr after incubation. On the other hand, aspirin was reported to be silylated almost instantaneously (4), and its trimethylsilyl derivative probably should be analyzed as soon as possible to reduce the extent of hydrolysis by the presence of moisture. If both salicylates are being analyzed simultaneously, a prescribed time for injection of the sample should be followed closely, recognizing the variables involved for optimizing each determination.

In conclusion, certain recommendations can be made pertaining to the routine assaying of salicylates by GLC. For the qualitative and quantitative analysis of the silylated derivative of salicylic acid, *N,N*-bis(trimethylsilyl)trifluoroacetamide is the silylating reagent of choice. Polyseal screw-capped stoppered tubes are also recommended as the reaction vessel due to their ease and reliability of use. In addition, injection should be performed between 1 and 4 hr after incubation for optimal and reliable results for salicylic acid.

REFERENCES

(1) L. J. Walter, D. F. Biggs, and R. T. Coutt, *J. Pharm. Sci.*, **63**, 1754(1974).

(2) M. Rowland and S. Riegelman, *ibid.*, **56**, 717(1967).

(3) S. Patel, J. H. Perrin, and J. J. Windheuser, *ibid.*, **61**, 1794(1972).

(4) B. H. Thomas, G. Solomonraj, and B. B. Coldwell, *J. Pharm. Pharmacol.*, **25**, 201(1973).

(5) R. C. Crippen and H. C. Freimuth, *Anal. Chem.*, **36**, 273(1964).

ACKNOWLEDGMENTS AND ADDRESSES

Received September 22, 1975, from the *Department of Chemistry, Philadelphia College of Pharmacy and Science, Philadelphia, PA 19104*

Accepted for publication December 10, 1975.

Supported by the Henry Leffmann Research Grant of the Wagner Free Institute of Science, Philadelphia.

The author expresses his appreciation for the criticism and help of Dr. Alfonso Gennaro.

High-Performance Liquid Chromatographic Separation of C-15-Epimers of 15-Methylprostaglandin E_2 Methyl Ester and 15-Methylprostaglandin E_2

R. K. LUSTGARTEN

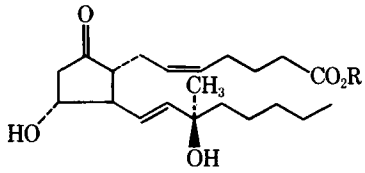
Abstract □ A high-performance liquid chromatographic system that permits quantitation of 15-methylprostaglandin E_2 and its methyl ester is described. Separation from the corresponding C-15 epimers is effected by adsorption chromatography on microparticulate silica with refractive index detection.

Keyphrases □ 15-Methylprostaglandin E_2 and methyl ester—high-performance liquid chromatographic analysis and separation from C-15-epimers □ High-performance liquid chromatography—analysis, 15-methylprostaglandin E_2 and methyl ester, separation from C-15-epimers □ Prostaglandins—15-methylprostaglandin E_2 and methyl ester, high-performance liquid chromatographic analysis and separation from C-15-epimers

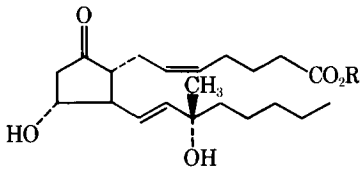
The methyl ester of 15-(*R*)-methylprostaglandin E_2 (I*a*) and its corresponding acid (I*b*) are potentially pharmacologically active in the treatment of gastric hyperacidity (1, 2). Procedures are known that would separate them from possible degradation products, *e.g.*, the prostaglandin A_2 and prostaglandin B_2 analogs (3–5), but there has been no efficient way of quantitating them in the presence of their 15-epimers (II*a* and II*b*). This paper presents a precise, rapid, and efficient method for doing so. The technique and data are discussed for the I*a*–II*a* system. Except for the composition of the mobile phase, the procedure and results for the I*b*–II*b* system directly parallel those for the esters, so this system will not be discussed in detail.

EXPERIMENTAL

Materials—Prostaglandin[1] samples, methyl acetate[2], and all other materials were obtained from commercial sources and used directly.



I*a*: R = CH_3
I*b*: R = H



II*a*: R = CH_3
II*b*: R = H

[1] The Upjohn Co.
[2] Fisher Certified grade.