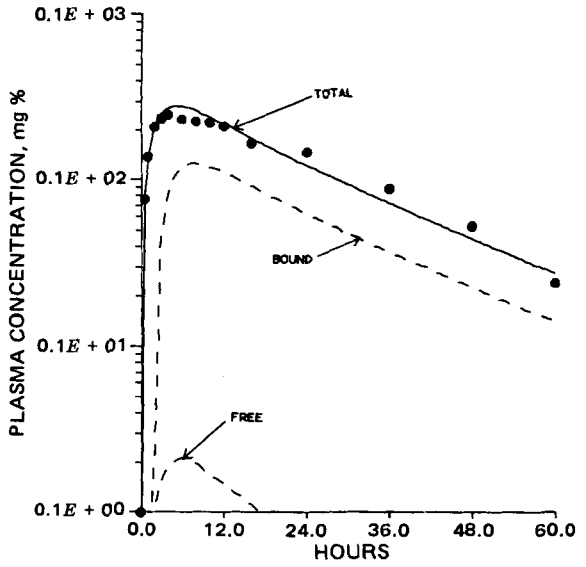

Figure 7—*Semilog plot of plasma concentration (●) versus time following oral sulfadimethoxine administration to one animal. Points were experimentally observed, and lines were calculated. The points and the solid line were multiplied by 2 to separate these values from the dashed lines representing free and bound drug concentrations.*

presented in Table IV. The lines in Figs. 4 and 7 were calculated using the best fit values of the parameters.

REFERENCES

(1) D. W. A. Bourne, R. F. Bevill, R. M. Sharma, R. P. Gural, and L. W. Dittert, *Am. J. Vet. Res.*, **38**, 967 (1977).

(2) R. F. Bevill, R. M. Sharma, S. H. Meachum, S. C. Wozniak, D. W. A. Bourne, and L. W. Dittert, *ibid.*, **38**, 973 (1977).

(3) G. D. Koritz, R. F. Bevill, D. W. A. Bourne, and L. W. Dittert, *ibid.*, **39**, 481 (1978).

(4) D. W. A. Bourne, L. W. Dittert, G. D. Koritz, and R. F. Bevill, *J. Pharmacokinet. Biopharm.*, **6**, 123 (1978).

(5) C. M. Stowe and C. S. Sisodia, *Am. J. Vet. Res.*, **24**, 525 (1963).

(6) R. Silvestri, P. Magnifico, and S. Glatstein, *ibid.*, **28**, 1783 (1967).

(7) H. G. Boxenbaum and S. A. Kaplan, *J. Pharmacokinet. Biopharm.*, **3**, 257 (1975).

(8) J. S. Annino, in "Standard Methods of Clinical Chemistry," vol. 3, Academic, New York, N.Y., 1961, pp. 200–206.

(9) E. Kruger-Thiemer, W. Diller, and P. Bunger, *Antimicrob. Agents Chemother.*, **5**, 183 (1965).

(10) C. M. Metzler, G. L. Elfind, and A. J. McEwen, "A User's Manual for NONLIN and Associated Programs, Research Biostatistics," Upjohn Co., Kalamazoo, Mich., 1974.

(11) K. Yamaki, T. Nakagawa, and T. Uno, *J. Pharmacokinet. Biopharm.*, **6**, 165 (1978).

(12) P.-L. Hsu, J. K. H. Ma, H. W. Jun, and L. A. Luzzi, *J. Pharm. Sci.*, **63**, 27 (1974).

(13) M. Nakagaki, N. Koga, and H. Terada, *Yakugaku Zasshi*, **84**, 516 (1964).

(14) I. Moriguchi, S. Wada, and J. Nishizawa, *Chem. Pharm. Bull.*, **16**, 601 (1968).

(15) M. S. Spector, "Handbook of Biological Data," Saunders, Philadelphia, Pa., 1956.

(16) E. Genazzani and G. Pagnini, *Am. J. Vet. Res.*, **24**, 1212 (1963).

ACKNOWLEDGMENTS

Supported by Food and Drug Administration Grant 223-74-178.

Quantitative Determination of Conjugated Estrogens in Formulations by Capillary GLC

G. K. PILLAI and K. M. McERLANE ˣ

Received December 15, 1980, from the *Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia, V6T 1W5 Canada.* Accepted for publication March 4, 1981.

Abstract □ A rapid capillary GLC method for the analysis of conjugated estrogen tablets and injectable formulations is described. The method involves the hydrolytic cleavage of the sodium sulfate ester conjugates by sulfatase enzyme. The free phenolic steroids are reacted sequentially with hydroxylamine hydrochloride and *N,O*-bis(trimethylsilyl)trifluoroacetamide. The resulting dual derivatives are analyzed on a 15-m glass capillary column wall coated with a cyanopropylmethyl silicone phase.

Keyphrases □ Estrogens, conjugated—quantitative determinations by capillary GLC □ GLC, capillary—quantitative determination of conjugated estrogens □ Steroids—conjugated estrogens, quantitative determination by capillary GLC

Conjugated estrogens is the official name (1) given to a group of closely related steroids derived from the urine of pregnant mares. These naturally occurring, water-soluble salts have been used since 1942 for the treatment of estrogen deficiency symptoms associated with menopause. Only recently, however, have the structures of these steroids been collectively reported (2) and quantitative methods developed (3, 4). For many years, the accepted method of analysis was that contained in USP XVIII (5), which specified colorimetric methods and limits for the major steroids, estrone and equilin, as well as for total estrogen content. An absorbancy ratio requirement was included in the identification tests for an unspecified steroid.

Recent revisions of the USP/NF (1) included a GLC identification test based on a published method (3) but retained the iron–kober chromogenic reagent for the assay of estrone, equilin, and total estrogen content. The identification test requires a prominent peak for one steroid, 17α-dihydroequilin, and this substance probably is the steroid monitored by the previous USP (5) identification test.

BACKGROUND

A consequence of the broad and nonquantitative specifications for the major and minor steroids is that compendial standards may not ensure pharmaceutical equivalence. More rigorous specifications for the indi-

0022-3549/81/0900-1072$01.00/0

Table I—Assay [a] of a Multiple-Injection Vial

Component	1	2	3	4	Mean ± *SD*	Percentage
17α-Estradiol	0.0210	0.0204	0.0209	0.0206	0.0207 ± 0.0002	2.82
17β-Estradiol	0.0072	0.0085	0.0143	0.0144	0.0111 ± 0.0037	1.51
17α-Dihydroequilin	0.1177	0.1209	0.1157	0.1244	0.1196 ± 0.0038	16.29
17β-Dihydroequilin	0.0076	0.0084	0.0084	0.0083	0.0081 ± 0.0003	1.10
Estrone	0.3472	0.3593	0.3519	0.3478	0.3515 ± 0.0055	47.87
Equilin	0.1626	0.1674	0.1675	0.1673	0.1662 ± 0.0024	22.64
17α-Dihydroequilenin	0.0130	0.0113	0.0098	0.0083	0.0106 ± 0.0020	1.44
17β-Dihydroequilenin	0.0014	0.0011	0.0005	0.0003	0.0008 ± 0.0005	0.11
Equilenin	0.0491	0.0463	0.0433	0.0431	0.0454 ± 0.0028	6.18
Total, mg	0.7268	0.7431	0.7323	0.7345	0.7342	
Total × 1.38, mg	1.0029	1.0254	1.0105	1.0136	1.0131	

[a] Amount is expressed as milligrams of each steroid in a 1.0-mg aliquot. Amount of conjugated estrogens found = 1.0131 ± 0.0093 mg.

vidual steroids are needed, especially in view of the widely varying potencies of each steroid in the mixture. For example, 17β-estradiol, although a minor component, exhibits 12 times the potency of the major steroid, estrone (6). Many methods have attempted to resolve all of the components of this relatively complex mixture, including paper chromatography (7–9), TLC (10–14), high-performance liquid chromatography (15–18), and GLC (2–4, 19–23). Of these, two GLC methods were capable of resolving most of the components and were applied toward quantitation of the pharmaceutical product (3, 4). However, both methods suffer from either long chromatographic times and poor column stability (3) or the need for dual injections and lack of resolution of 17β-estradiol from 17α-dihydroequilin (4). In addition, the quantitation of the estrogens was further hindered by the presence of an impurity subsequently identified as equol (21). This impurity, if not removed prior to analysis, would interfere with both GLC methods (3, 4). This interference has necessitated the removal of this impurity by a lengthy extraction step (4).

As an alternative to packed column chromatographic analysis of the equine estrogens, capillary columns were recently used (24). However, the method did not significantly improve the resolution of estrone and equilin and did not address the problem of resolution of 17β-estradiol from 17α-dihydroequilin. Similar efforts in this laboratory resulted in the complete resolution of all known equine estrogens present in conjugated estrogens using a highly polar capillary column (25). The present study was based on the capillary column separation reported earlier (25) but included a quantitative determination of all known estrogens as well as a reported impurity, equol, in tablet and injectable formulations.

EXPERIMENTAL

A gas chromatograph[1] equipped with a capillary inlet system, a flame-ionization detector, and a data terminal[2] was used for all measurements.

A commercial 15-m × 0.25-mm borosilicate column[3] wall coated with cyanopropylmethyl silicone liquid phase (Silar 10C) was used with a helium carrier gas flow of 0.8 ml/min through the column and a split vent flow of 40 ml/min. The column inlet pressure was 10 psi. The injector and detector temperatures were both 250°; the oven temperature was programmed from 170° for 7 min, heated at 2.3°/min to 220°, and maintained at this temperature for 5 min.

GLC–mass spectral data[4] were obtained with electron-impact ionization at 70 ev.

Materials—Reagent grade chloroform, acetic acid, sodium acetate, and anhydrous sodium sulfate were used. *N,O*-Bis(trimethylsilyl)trifluoroacetamide[5] and pyridine[5] were silylation grade. Sulfatase enzyme[6], ethinyl estradiol[7], hydroxylamine hydrochloride[8], and equine estrogens[9] were used as received.

Sample Extraction and Preparation—*Tablets*—Twenty tablets were weighed and powdered, and an amount equivalent to 1 mg of conjugated estrogens was weighed into a 50-ml, polytef-lined, screw-capped culture tube. To this tube was added 15 ml of an acetate buffer (0.02 *M*, pH 5.2), and the tube was shaken mechanically for 20 min. An amount equivalent to 2000 units of sulfatase enzyme was added, and the contents were incubated in a water bath at 45° for 30 min with occasional shaking. Then 0.2 ml of the internal standard, ethinyl estradiol, was added in 10 ml of chloroform, and the tube was shaken mechanically for an additional 30 min.

After centrifugation at 2000 rpm for 15 min, the chloroform layer was separated as completely as possible and filtered through a bed of anhydrous sodium sulfate. The filtrate, 3 ml at a time, was evaporated to dryness under a clean nitrogen stream in a 5-ml conical vial[10]. To the residue was added 200 μl of a 2% solution of hydroxylamine hydrochloride in dry pyridine. The polytef-lined screw-capped vial was heated in an aluminum block[11] at 70° for 30 min. The excess pyridine in the vial was evaporated under a gentle stream of clean, dry nitrogen, 150 μl of *N,O*-bis(trimethylsilyl)trifluoroacetamide and 50 μl of dry pyridine were added, and the vial was heated at 70° for an additional 10 min. Then 2 μl of the resulting solution was injected directly into the gas chromatograph. Peak identification was confirmed by comparison of retention times with those of pure standards.

Injectable Formulation—The contents of a 25-mg multiple injection vial were dissolved in an acetate buffer and brought to volume in a 50-ml volumetric flask with acetate buffer. An aliquot equivalent to 1 mg of conjugated estrogens was processed as described.

RESULTS AND DISCUSSION

Conjugated estrogens exist in the pharmaceutical product as the sodium salts of the sulfate esters. For GLC analysis, it was necessary to hydrolyze these esters to their free phenolic forms. Two hydrolysis methods were used previously. The first method (2) involved the use of hydrochloric acid at elevated temperature, and the second consisted of

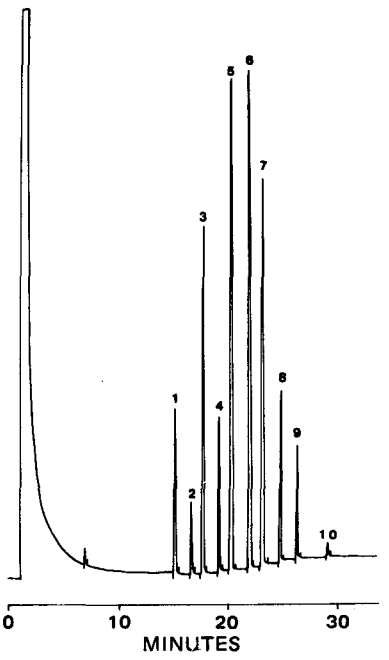

Figure 1—*Chromatogram of a standard mixture of equine estrogens as their oxime–trimethylsilyl derivatives. Key: 1, 17α-estradiol; 2, 17β-estradiol; 3, 17α-dihydroequilin; 4, 17β-dihydroequilin; 5, ethinyl estradiol (internal standard); 6, estrone; 7, equilin; 8, 17α-dihydroequilenin; 9, 17β-dihydroequilenin; and 10, equilenin.*

[1] Model 5830 A, Hewlett-Packard, Avondale, Pa.
[2] Model 18850A, Hewlett-Packard, Avondale, Pa.
[3] Alltech Associates, Rockford, Ill.
[4] Varian Mat 111 mass spectrometer, Hewlett-Packard 5700A gas chromatograph, and Varian 621/L computer.
[5] Pierce Chemical Co., Rockford, Ill.
[6] Type H-2, Sigma Chemical Co., St. Louis, Mo.
[7] Sigma Chemical Co., St. Louis, Mo.
[8] British Drug Houses, London, England.
[9] Ayerst Pharmaceuticals, Montreal, Canada.
[10] Reacti-Vial, Pierce Chemical Co., Rockford, Ill.
[11] Reacti-Therm heating module, Pierce Chemical Co., Rockford, Ill.

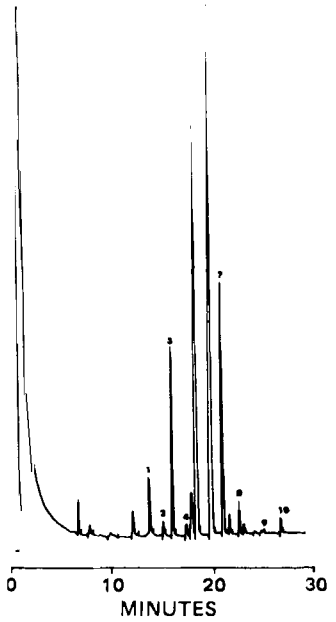

Figure 2—*Chromatogram of equine estrogens as their oxime–trimethylsilyl derivatives derived from a multiple-injection vial. Key: 1, 17α-estradiol; 2, 17β-estradiol; 3, 17α-dihydroequilin; 4, 17β-dihydroequilin; 5, ethinyl estradiol (internal standard); 6, estrone; 7, equilin; 8, 17α-dihydroequilenin; 9, 17β-dihydroequilenin; and 10, equilenin.*

incubation with sulfatase enzyme (3). The use of hydrochloric acid to hydrolyze steroid sulfates is known to cause steroid transformations (26, 27); and since it was considered essential to measure the original components of the mixture without creating artifacts during workup, acid hydrolysis could not be used. Although optimum conditions for the use of the sulfatase enzyme were established previously (4), it was considered necessary to ascertain that phosphates, suspected to be present in one formulation, did not inactivate the enzyme. Accordingly, a series of enzyme concentrations was incubated with identical aliquots of this tablet formulation, and an optimum enzyme quantity of 2000 units was found to yield maximum estrogen quantities. Above this value, no increase in estrogen levels was noted.

A prior examination of derivative combinations and capillary column phases led to the development of an excellent separation of all known equine estrogens as a dual derivative (25). Among the derivatives evaluated, an oxime–trimethylsilyl combination was most suitable.

As shown in Fig. 1, the chromatogram obtained for the oxime–trimethylsilyl derivatives exhibited excellent resolution of the equine estrogens. The potent steroid, 17β-estradiol, was well resolved from 17α-dihydroequilin, as was estrone from equilin. These two steroid pairs presented the greatest problems in previous chromatographic methods. In addition, the total chromatographic time was shortened as compared to the values, 55 (4) and 70 (3) min, reported earlier.

The chromatographic properties of samples obtained from hydrolysis of commercial formulations of conjugated estrogens are shown in Figs.

Figure 3—*Chromatogram of equine estrogens as their oxime–trimethylsilyl derivatives derived from a 2.5-mg tablet. Key: 1, 17α-estradiol; 2, 17β-estradiol; 3, 17α-dihydroequilin; 4, 17β-dihydroequilin; 5, ethinyl estradiol (internal standard); 6, estrone; 7, equilin; 8, 17α-dihydroequilenin; 9, 17β-dihydroequilenin; 10, equilenin; 11, equol; and 12, ethyl phthalate.*

Table II—Composition of Commercial Formulations Containing Conjugated Estrogens [a]

Component	Sample: 25-mg Multiple-Injection Vial	Sample: 2.5-mg Tablet
17α-Estradiol	2.82	0.66
17β-Estradiol	1.06	0.12
17α-Dihydroequilin	16.23	2.87
17β-Dihydroequilin	1.11	0.23
Estrone	48.07	62.01
Equilin	22.45	26.95
17α-Dihydroequilenin	1.65	1.12
17β-Dihydroequilenin	0.11	0.12
Equilenin	6.49	6.51
Equol	—	3.85

[a] These results represent the percentage of each conjugated estrogen; each value is the average of two assays. The formulations are from two different manufacturers.

2 and 3. Unlike the chromatogram shown in Fig. 1 and those reported for the commercial products using conventional packed columns, these chromatograms exhibit evidence of several additional minor components. One may be 9-dehydroestrone; however, a reference standard for this material was not available for comparison. The two commercial formulations display significant qualitative and quantitative differences. The formulation shown in Fig. 3 contained ethyl phthalate (retention time, 7.32 min) and equol (retention time, 22.07 min). The identities of these two extraneous substances were confirmed by comparison of their GLC and mass spectral characteristics. Ethyl phthalate is a probable residue from the tablet film-coating process, while equol was previously reported (21) as a contaminant of equine estrogens in some formulations. Since the present method can resolve equol from the equine steroids, prewash of the unhydrolyzed sample with benzene (4) is unnecessary since equol will not interfere with quantitation. Elimination of this purification step further reduces the total analysis time.

The subsequent quantitation of the separated equine estrogens was accomplished by determining the relative response ratios of samples of each individual equine estrogen as compared to the internal standard, ethinyl estradiol. At least six aliquots of each steroid, at levels bracketing the anticipated quantity in the mixture, were analyzed with a constant amount of internal standard. For all determinations, the correlation between the peak area ratios and concentrations was ≥0.998. The precision of the assay was checked by duplicate injections of four aliquots taken from a single 50-ml solution prepared from a multiple-injection vial. Each of the four aliquots was separately hydrolyzed, extracted, derivatized, and assayed. The mean and standard deviation of each steroid and the total are given in Table I.

Quantitation of conjugated estrogens in some available formulations provided the data shown in Table II; unlike with previous methods (3, 4), the quantity of 17β-estradiol now can be determined with certainty. In addition, it was found that the quantity of equilenin was double the amounts reported earlier, perhaps because of the increase in sensitivity due to the shorter retention time in the present chromatographic assay.

REFERENCES

(1) "The United States Pharmacopeia," 20th rev., United States Pharmacopeial Convention, Rockville, Md., 1980, p. 298.

(2) R. Roman, C. A. Yates, J. F. Miller, and W. J. A. VandenHeuvel, *Can. J. Pharm. Sci.*, **10**, 8 (1974).

(3) R. Johnson, R. Masserano, R. Haring, B. Kho, and G. Schilling, *J. Pharm. Sci.*, **64**, 1007 (1975).

(4) K. M. McErlane and N. M. Curran, *ibid.*, **66**, 523 (1977).

(5) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970.

(6) O. Gisvold, in "Textbook of Organic Medicinal and Pharmaceutical Chemistry," 3rd ed., C. O. Wilson and O. Gisvold, Eds., Lippincott, Philadelphia, Pa., 1956, p. 578.

(7) E. Heftmann, *Science*, 111, 571 (1950).

(8) J. E. Cox, *J. Chromatogr.*, **61**, 193 (1971).

(9) E. Heftmann, *J. Am. Chem. Soc.*, **73**, 851 (1951).

(10) G. J. Kroll, G. R. Boyden, R. H. Moody, J. C. Comeau, and B. T. Kho, *J. Chromatogr.*, **61**, 187 (1971).

(11) K. M. Rajkowski and G. D. Broadhead, *ibid.*, **89**, 374 (1974).

(12) L. E. Crocker and B. A. Lodge, *ibid.*, **62**, 158 (1971).

(13) *Ibid.*, **69**, 419 (1978).

(14) I. Schroeder, G. Lopez-Sanchez, J. C. Medina-Acevedo, and M. del Carmen Espinosa, *J. Chromatogr. Sci.*, **13**, 37 (1975).
(15) A. G. Butterfield, B. A. Lodge, and N. J. Pound, *ibid.*, **11**, 401 (1973).
(16) R. W. Roos, *ibid.*, **14**, 505 (1976).
(17) G. Capitano and R. Tscherne, *J. Pharm. Sci.*, **68**, 311 (1979).
(18) P. I. Mussey, D. C. Collins, and J. R. K. Prudy, *Steroids*, **31**, 583 (1978).
(19) I. Schroeder, J. C. Medina-Acevedo, and G. Lopez-Sanchez, *J. Chromatogr. Sci.*, **10**, 183 (1972).
(20) K. M. McErlane, *ibid.*, **12**, 97 (1974).
(21) G. Carrignan, N. M. Curran, B. A. Lodge, and K. M. McErlane, *Can. J. Pharm. Sci.*, **13**, 73 (1978).
(22) R. N. Johnson, R. P. Masserano, B. T. Kho, and W. P. Adams, *J. Pharm. Sci.*, **67**, 1218 (1978).
(23) W. P. Adams, J. Hasegawa, R. N. Johnson, and R. C. Haring, *ibid.*, **68**, 986 (1979).
(24) J. S. Zweig, R. Roman, W. B. Hagerman, and W. J. A. VandenHeuvel, *HRC CC, J. High Resolut. Chromatogr. Chromatogr. Commun.*, **3**, 169 (1980).
(25) G. K. Pillai and K. M. McErlane, *ibid.*, **4**, 70 (1981).
(26) C. A. Muehlbaecher and E. K. Smith, *Clin. Chem.*, **16**, 158 (1970).
(27) L. P. Cawley, B. O. Musser, W. Faucette, S. Beckloff, and H. Learned, *ibid.*, **11**, 1009 (1965).

ACKNOWLEDGMENTS

Supported by a grant from the Natural, Applied and Health Sciences, University of British Columbia.

The authors thank Ayerst Pharmaceuticals, Montreal, Canada, for their gift of equine estrogens, and Dr. B. Lodge, Health Protection Branch, Ottawa, Canada, for a reference sample of equol. The authors are grateful to Mr. R. Burton for the mass spectral analysis.

Thiourea and Thiosemicarbazide Derivatives Structurally Related to Hexestrol: Synthesis and Anticancer and Other Pharmacological Properties

A.-MOHSEN M. E. OMAR ‡x, A. M. FARGHALY *, A. A. B. HAZZAI *, N. H. ESHBA *, F. M. SHARABI ‡, and T. T. DAABEES ‡

Received May 16, 1980, from the **Department of Pharmaceutical Chemistry and the* ‡*Department of Pharmacology, Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt.* Accepted for publication November 24, 1980.

Abstract □ Two novel series of thio compounds bearing internal structural modifications of hexestrol were synthesized as potential anticancer agents. The first contains several *N*-substituted thiourea functions, and the second contains various N^4-substituted-3-thiosemicarbazide moieties in place of one α-ethyl group of hexestrol dimethyl ether. The products showed no antileukemic activity in the P-388 lymphocytic leukemia system and did not exhibit any anticonvulsant or estrogenic properties.

Keyphrases □ Hexestrol—structurally related thiourea and thiosemicarbazide derivatives as anticancer agents □ Anticancer drugs, potential—compounds related to hexestrol, screened against P-388 lymphocytic leukemia □ Thioureas—synthesis, hexestrol derivatives, evaluation of anticonvulsant and anticancer activities □ Thiosemicarbazide—synthesis of hexestrol derivatives, evaluation of anticonvulsant and anticancer activities

Concurrent with ongoing studies of the cyclodesulfurization of thio compounds into various heterocyclic derivatives (1), related studies have been concerned with the synthesis of thio compounds derived from menadione (2), phthiocol (2), steroids (3), diethylstilbestrol (4), and theophylline (5), the biologically active nuclei, for various pharmacological purposes. Extending these studies to compounds containing thio functions as internal modifications of hexestrol, the thio derivatives IV–XI and XIV–XVIII (Scheme I) were prepared and tested for anticancer, estrogenic, and anticonvulsant activities.

RESULTS AND DISCUSSION

Chemistry—1,2-Bis(*p*-methoxyphenyl)butylamine (II), required as the starting material, was prepared through conversion of α-ethyldesoxyanision (I) into the corresponding oxime (6), using hydroxylamine hydrochloride and potassium acetate in ethanol, followed by reduction of the product with aluminum amalgam in aqueous ethanol (7). The amine (II) was reacted with the equivalent amount of alkyl-, aryl-, or aralkylisothiocyanates (III) in refluxing ethanol to give *N*-[1,2-bis(*p*-methoxyphenyl)butyl]-*N'*-substituted thioureas (IV–XI) in high yields (Table I).

The treatment of the amine (II) with ethyl bromoacetate and sodium carbonate in anhydrous acetone gave the glycinate ester (XII), which was heated with excess hydrazine hydrate to yield the N^{α}-[1,2-bis(*p*-methoxyphenyl)butyl]-α-aminoacetohydrazide (XIII). Heating equimolar amounts of this acid hydrazide and the selected isothiocyanate derivatives (III) in refluxing ethanol gave the required 4-substituted-1-{N^{α}-[1,2-bis(*p*-methoxyphenyl)butyl]-α-aminoacetyl}-3-thiosemicarbazides (XIV–XVIII) (Scheme I and Table I). The products were identified by the appearance of four bands at 1550–1525, 1345, 1320–1305, and 945–910 cm^{-1}, characteristic for the –N–C=S amides of I, II, III, and IV, respectively, in the IR spectra (5, 8).

The PMR spectra of representative examples of the thiourea derivatives IV, VIII, and IX and the thiosemicarbazides XIV, XV, and XVII showed the common protons resonating at various shifts (Table II). In addition to these signals, the other NH proton of the thiourea part was identified at various shifts depending on the substituent present. It appeared as a triplet at δ 5.90 ppm for the allyl derivative (IV), as a singlet at δ 8.11 ppm for the *m*-tolyl derivative (VIII), and as a multiplet at δ 6.11 ppm for the benzyl thiourea (XI). Likewise, the N^4-H proton of the thiosemicarbazides appeared as a broad multiplet at δ 6.59 and 6.54 ppm for XIV and XV, respectively, and as a singlet at δ 8.57 ppm for XVII.

The mass spectrum of *N*-[1,2-bis(*p*-methoxyphenyl)butyl]-*N'*-benzylthiourea (XI) did not show the molecular ion peak at *m/z* 434. However, it indicated that the compound had undergone fragmentation through two pathways (Scheme II). The first pathway produced ions A and B at *m/z* 284 and 150, while the second pathway gave ions C and D at *m/z* 269 and 165, respectively. These four ions, on further fragmentation, produced various daughter ions (Scheme II), of which the tropylium ion at *m/z* 91 was almost as intense as the base peak at *m/z* 78. The mass spectrum of the thiosemicarbazide (XVII) did not show the molecular ion peak at *m/z* 506, but it showed the base peak at *m/z* 208 (see *Experimental*).

0022-3549/81/0900-1075$01.00/0