volatility, being readily evaporated prior to the derivatization step. Using a V_{org}/V_{aq} ratio of 4.98 ± 1% of busulfan (n = 5) was extracted from plasma (busulfan concentration 50 ng/ml).

GC of Intact Busulfan—The peak height ratios of busulfan to 9-bromophenanthrene obtained after injections of different amounts of the compounds at two column temperatures were drastically reduced when lower amounts of busulfan were chromatographed; in all cases poor precision was obtained (Table II). Analysis of busulfan at the lower temperature (175°), keeping the retention time the same by adjustment of the carrier gas flow, gave the highest peak height ratio, which indicates that the results obtained are primarily due to degradation of busulfan in the chromatographic system and not to adsorption phenomena (6). This assumption is also supported by the fact that when using different batches of OV-17 column packing material, multiple asymmetric peaks were occasionally observed.

Conversion of Busulfan to 1,4-Diiodobutane—The reaction of busulfan with sodium iodide proceeds according to Scheme I. The reaction was performed in acetone since nucleophilic substitution reactions are known to be rapid in this solvent (7). The time course for busulfan, I, and 1,4-diiodobutane using 1 M sodium iodide in acetone is given in Fig. 1. Evaluation of the apparent first-order rate constants by nonlinear regression analysis gave $k_1 = 0.266 \pm 0.013$ and $k_2 = 0.124 \pm 0.008$ min^{-1}. Since the ratio between the constants is ~2, it follows that the methanesulfonate ester group of busulfan and that of I have similar reactivity (3). A quantitative reaction was obtained after 20 min using 1 M sodium iodide and a temperature of 70°.

Chromatographic Properties—1,4-Diiodobutane had excellent GC properties giving a symmetric peak (Fig. 2). No indications of decomposition in the chromatographic system were observed.

Detection, Selectivity, and Precision—The minimum detectable concentration (MDC) value obtained by electron-capture detection (ECD) was ~10 times lower than that obtained by selected-ion monitoring (SIM), 0.6×10^{-16} and 5.7×10^{-16} mole/sec, respectively[5]. However, analysis of plasma samples revealed that the higher sensitivity of the ECD could not be utilized because of interfering peaks in the chromatograms. The blanks varied considerably between patients and, in most cases, it was not possible to perform quantitations <10 ng/ml. Since the plasma peaks after administration of therapeutic doses (2 mg) of busulfan are 20–30 ng/ml, meaningful pharmacokinetic studies require determinations in the low nanogram range. A chromatogram obtained from plasma using SIM is given in Fig. 2. The standard curve obtained from plasma using SIM was linear within the range studied (10–400 ng/ml). A least-squares analysis gave a correlation coefficient of 0.9997, a slope of $2.12 \times 10^{-2} \pm 0.03 \times 10^{-2}$, and an intercept of $4.7 \times 10^{-2} \pm 7.6 \times 10^{-2}$. The relative standard deviation was ±2.6% at 100 ng/ml and ±4.3% at 10 ng/ml (n = 5).

[5] Signal to noise ratio = 3. ECD: A Varian ^{63}Ni-detector (DC) operating at a foil temperature of 200°. SIM: LKB 2091, focusing at m/z 183 (70 eV).

REFERENCES

(1) M. V. Nadkarni, E. G. Trams, and P. K. Smith, *Cancer Res.*, **19**, 713 (1959).

(2) H. Vodopick, H. E. Hamilton, and H. L. Jackson, *J. Lab. Clin. Med.*, **73**, 266 (1969).

(3) R. F. Hudson, G. M. Timmis, and R. D. Marshall, *Biochem. Pharmacol.*, **1**, 48 (1958).

(4) H. Ehrsson, U. Lönroth, I. Wallin, M. Ehrnebo, and S. O. Nilsson, *J. Pharm. Pharmacol.*, **33**, 313 (1981).

(5) H. Ehrsson and U. Lönroth, *J. Pharm. Sci.*, **71**, 826 (1982).

(6) K. Grob, *J. High Resol. Chromatogr.*, **3**, 585 (1980).

(7) A. J. Parker, *Chem. Rev.*, **69**, 1 (1969).

ACKNOWLEDGMENTS

The authors thank Dr. S. O. Nilsson for performing the nonlinear regression analysis.

Steroidal Thiourea and Thiazoline Derivatives: Synthesis and *In Vitro* Effects on Bovine Pancreatic Ribonuclease Activity

EL-SEBAII A. IBRAHIM *, A.-MOHSEN M. E. OMAR *x, N. S. HABIB *, OMAIMA M. ABOULWAFA *, S. M. EL-SEWEDY ‡, and J. BOURDAIS §

Received February 24, 1982, from the **Pharmaceutical Chemistry Department, Faculty of Pharmacy*, the ‡*Department of Applied Medical Chemistry, Medical Research Institute, University of Alexandria, Egypt* and the §*Laboratorie de Chimie des Hétérocycles d'Intérêt Biologique, Université d'Aix-Marseille II, Faculté de Medecine Nord, 13326 Marseille, Cédex 15, France.* Accepted for publication August 16, 1982.

Abstract □ Two novel series of steroidal derivatives containing various thiourea and substituted thiazoline moieties attached to the 2- or 4-position of estrone were synthesized and examined for *in vitro* effect on bovine pancreatic ribonuclease activity. All compounds studied exhibited a catabolic activity. The steroidal thiazoline derivatives were more potent activators of ribonuclease than the steroidal thioureas.

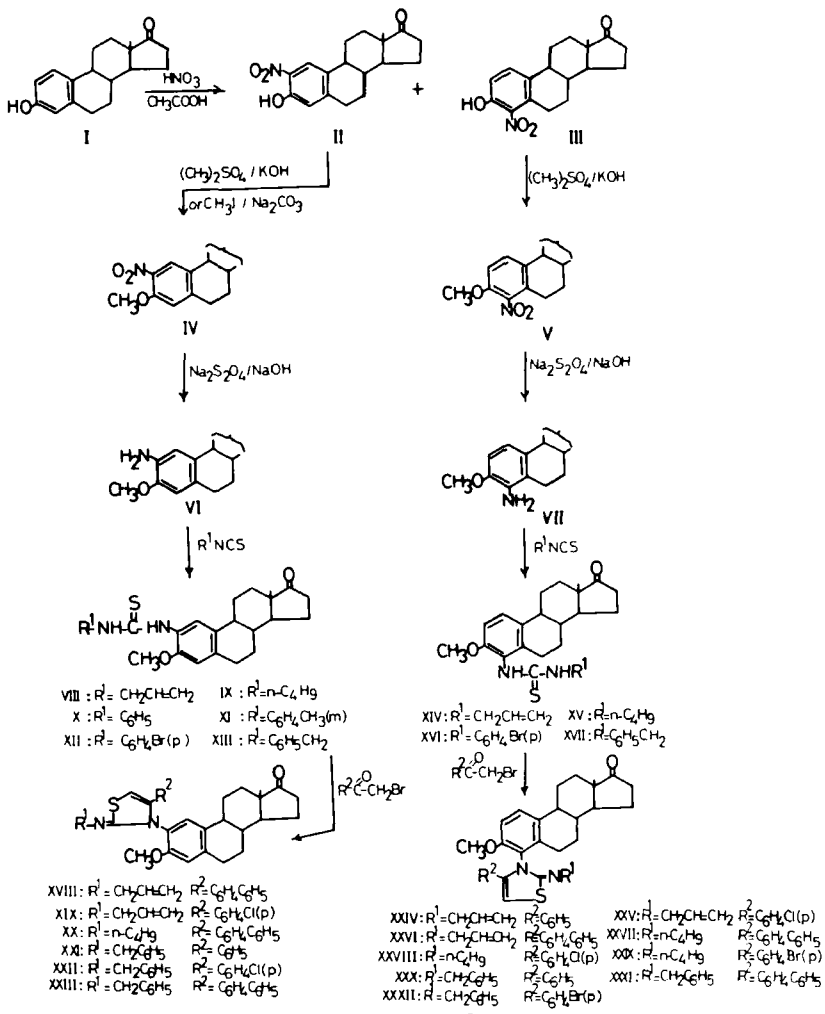
Keyphrases □ Steroids—thiourea and thiazoline derivatives, synthesis, *in vitro* effect on bovine pancreatic ribonuclease activity □ Synthesis—steroidal thiourea and thiazoline derivatives, *in vitro* effect on bovine pancreatic ribonuclease activity □ Catabolic activity—steroidal thiourea and thiazoline derivatives, *in vitro*, bovine pancreatic ribonuclease activity

Recent reports from this laboratory have described the synthesis and pharmacological properties of a variety of androgenic and estrogenic thiosemicarbazones (1), acylhydrazones (2–5), and several steroidal heterocycles (6, 7). Further interest in structure–activity relationships (SAR) of steroidal heterocyclics prompted the preparation of XVIII–XXXII (Scheme I) to evaluate the changes in the endocrinological activity caused when the 2- or 4-position of estrone-3-methyl ether is blocked by variously substituted thiazoline moieties. Some of the steroidal thioureas (VIII–XVI), prepared as starting materials, and the thiazoline derivatives were found to possess catabolic-like properties as indicated from their *in vitro* effect on the activity of bovine pancreatic ribonuclease.

RESULTS AND DISCUSSION

Synthesis—The designed compounds (XVIII–XXXII) were prepared in accordance with the sequence of reactions shown in Scheme I. The 2-

0022-3549/83/1000-1205$01.00/0



Scheme I

and 4-mononitroestrones (II and III), prepared by nitration of estrone (I) with a mixture of nitric acid and acetic acid (8), were methylated to the corresponding 3-methyl ethers (IV and V) using dimethyl sulfate and potassium hydroxide (9) or methyl iodide and sodium carbonate (10). The products were reduced by sodium dithionite in alkaline medium (9) to give the required 2-amino- (VI) and 4-aminoestrone-3-methyl (VII) ethers in good yields.

Treatment of the amines (VI and VII) with the appropriate alkyl, aryl, or aralkylisothiocyanate derivatives in absolute ethanol (11) gave, respectively, the *N*-substituted *N'*-(3-methoxy-17-oxoestra-1,3,5(10)-trien-2-yl)thioureas (VIII–XIII) and *N*-substituted *N'*-(3-methoxy-17-oxoestra-1,3,5(10)-trien-4-yl)thioureas (XIV–XVII) (Table I). The reaction of VIII, IX, XIII–XV, and XVII, in which R^1 was an alkyl or aralkyl function, with the selected phenacyl bromide in refluxing absolute ethanol (12) proceeded smoothly and gave high yields of the required 2-(2',4'-disubstituted thiazolin-3'-yl)-3-methoxyestra-1,3,5(10)-trien-17-ones (XVIII–XXIII) and the corresponding 4-(2',4'-disubstituted thiazolin-3'-yl)-3-methoxyestra-1,3,5(10)-trien-17-ones (XXIV–XXXII) (Table II). When R^1 was an aryl moiety, as in X–XII and XVI, the reaction with phenacyl bromide gave a mixture of two products that could not be separated. In addition, in one case, when IX was treated with *p*-chlorophenacyl bromide, the *S*-alkylpseudothiourea hydrobromide salt (XXXIII) was obtained (Scheme II).

The products were identified by elemental analyses, IR, UV, and ^{1}H-NMR spectra and, for representative examples, by mass spectra (Tables I, II, and III). The UV spectra showed two absorption maxima at 245–253 and 290–295 nm for VIII and XIII, a single absorption maximum at 276–286 nm for X–XII, and two absorption maxima at 244–251 and 277–282 nm for the thioureas XIV–XVII. The steroidal thiazolines, on the other hand, showed one absorption maximum at ~295 nm for XIX and XXI, while XXV and XXX absorbed at 288 nm. The remainder of the thiazoline derivatives showed two absorption maxima, sometimes as a shoulder, at 256–266 and 290–320 nm. The addition of hydrochloric acid caused a hypsochromic shift of the absorption maxima of all thiazoline derivatives (Tables I and II).

^{1}H-NMR spectra of the steroidal thiourea derivatives (Table I) showed the signals for the common protons of the C_{18}—CH_3, C_3—OCH_3, C_1—H, C_2—H, and C_4—H of the steroidal nucleus (13) at the expected chemical shift. The N—H proton attached to ring A of estrone resonated downfield between 7.25 and 7.97 ppm and was shown as a singlet disappearing on deuteration. The chemical shift of the other N—H proton was dependent on the nature of the substituent present. In the allyl (VIII and XIV), butyl (IX and XV), and benzyl (XIII and XVII) derivatives, the proton was shown at a multiplet at 5.65–6.25 ppm, while in the *p*-bromophenylthiourea (XII), it appeared as a singlet at 7.88 ppm. The ^{1}H-NMR spectra of the steroidal thiazolines (XVIII–XXXI), on the other hand, lacked the signals due to N—H protons, but showed a singlet between 5.72 and 5.83 ppm for the thiazoline C_5—H (13). The chemical shifts of the other protons were almost the same as those of the thiourea derivatives (Table III).

The ^{1}H-NMR spectrum of the steroidal pseudothiourea derivative (XXXIII) did not show the signal of the butyl-NH proton, indicating that this proton was involved in the enolization of the thiourea (IX) from the thione to the thiol form. In addition, the absence of a distinct signal for the two methylenic protons of the S—CH_2—CO function suggested that enolization of XXXIIIa to XXXIIIb had taken place (Scheme II). As a result, the olefinic C—H proton has been identified at low field at ~6.56 ppm while the OH proton was included in the fingerprint area of the steroidal skeleton.

The mass spectra of XV and XVII, as representative examples of the synthesized thioureas, showed the molecular ion peak at *m/z* 414 and 448, respectively (Scheme III). A common fragmentation pattern of these compounds was found to be the elimination of the 3-OCH_3 group followed by cyclization of the produced ion to give the base peak A at *m/z* 383 for the butyl derivative and 417 for the benzyl derivative (Scheme III, Pathway 1). In accordance with Pathway 2, the compounds underwent

Table I—Synthesized *N*-Substituted *N'*-(3-Methoxy-17-oxoestra-1,3,5(10)-trien-2-yl)thioureas (VIII–XIII) and *N*-Substituted *N'*-(3-Methoxy-17-oxoestra-1,3,5(10)-trien-4-yl)thioureas (XIV–XVII)

Compound	Reaction Conditions	Yield, %	Melting Point[a]	Molecular Formula	Analysis, %	Calc.	Found	UV ethanol λ_{max} (log ϵ)	^{1}H-NMR (δ), ppm
VIII	Reflux (1 hr)	95	169–171°	$C_{23}H_{30}N_2O_2S$	C	69.32	68.99	247 (4.212), 291 (3.892)	0.9 (s, 3, C_{18}—CH_3), 3.8 (s, 3, OCH_3), 4.28 (m, 2, allyl H), 5.09 (m, 1, allyl H), 5.23 (m, 1, allyl H), 5.7–6.2 (group of singlets, 2, allyl H and NH, disappearing on deuteration), 6.69 (s, 1, C_4—H), 7.24 (s, 1, C_1—H), 7.97 (s, 1, steroidal NH, disappearing on deuteration)
					H	7.59	7.76		
					N	7.03	6.60		
IX	Reflux (1 hr)	90	101–103°	$C_{24}H_{34}N_2O_2S$	C	69.53	69.50	245 (4.273), 290 (3.968)	0.89 (s, 3, C_{18}—CH_3), 0.90 (t, 3H, J = 6 Hz, CH_2CH_3), 3.59 (m, 2, CH_2—CH_2NH), 3.78 (s, 3, OCH_3), 6.04 (m, 1, CH_2NH, disappearing on deuteration), 6.66 (s, 1, C_4—H), 7.19 (s, 1, C_1—H), 7.4 (s, 1, steroidal NH, disappearing on deuteration)
					H	8.27	8.30		
					N	6.76	6.70		
X	Room temperature (overnight)	90	169–171°	$C_{26}H_{30}N_2O_2S$	C	71.86	71.50	276 (4.285)	
					H	6.98	7.10		
					N	6.45	6.30		
XI	Room temperature (overnight)	93	166–168°	$C_{27}H_{32}N_2O_2S$	C	72.29	72.10	279 (4.324)	
					H	7.19	7.10		
					N	6.25	6.50		
XII	Reflux (30 min)	87	193–195°	$C_{26}H_{29}BrN_2O_2S$	C	60.81	61.14	286(4.279)	0.87 (s, 3, C_{18}—CH_3), 3.77 (s, 3, OCH_3), 6.63 (s, 1, C_4—H), 7.2 (s, 1, C_1—H), 7.25 (d, 2, J = 9 Hz, aromatic H), 7.48 (d, 2, J = 9 Hz, aromatic H), 7.88 (s, 1, BrC_6H_4NH, disappearing on deuteration), 7.92 (s, 1, steroidal NH, disappearing on deuteration)
					H	5.65	6.01		
					N	5.45	5.10		
XIII	Reflux (10 min)	90	152–154°	$C_{27}H_{32}N_2O_2S$	C	72.29	72.17	253 (4.233), 295 (3.893)	0.82 (s, 3, C_{18}—CH_3), 3.73 (s, 3, OCH_3), 4.8 (t, 2H, J = 5 Hz, $CH_2C_6H_5$, becoming a doublet at 4.85 on deuteration), 6.25 (m, 1, $C_6H_5CH_2NH$, disappearing on deuteration), 6.61 (s, 1, C_4—H), 7.12 (s, 1, C_1—H), 7.28 (s, 5, aromatic H), 7.52 (s, 1, steroidal NH, disappearing on deuteration)
					H	7.19	7.13		
					N	6.25	6.38		
XIV	Reflux (3.5 hr)	98	181–183°	$C_{23}H_{30}N_2O_2S$	C	69.32	69.40	251 (4.253), 282 (3.609)	0.9 (s, 3, C_{18}—CH_3), 3.80 (s, 3, OCH_3), 4.26 (m, 2, allyl H), 5.04 (m, 1, allyl H), 5.16 and 5.22 (2 m, 1, allyl H), 5.65–6.06 (m, 2, allyl H+, CH_2NH, becoming a group of singlets at 5.69–6.14 for allyl H after deuteration), 6.82 (d, 1, J = 9 Hz, C_2—H), 7.3 (d, 2, J = 9 Hz, C_1—H + steroidal NH, becoming 1H after deuteration)
					H	7.59	7.67		
					N	7.03	7.20		
XV	Reflux (3 hr)	98	162–164°	$C_{24}H_{34}N_2O_2S$	C	69.53	69.58	245 (4.331), 281 (3.707)	0.88 (t, 3, J = 6 Hz, CH_2CH_3), 0.89 (s, 3, C_{18}—CH_3), 3.54 (q, 2, J = 6 Hz and 12 Hz, $NHCH_2$, becoming a triplet on deuteration at 3.58, J = 6 Hz), 3.77 (s, 3, OCH_3), 5.67 (t, 1, J = 6 Hz, CH_2NH, disappearing on deuteration), 6.8 (d, 1, J = 9 Hz, C_2—H), 7.27 (d, 2, J = 9 Hz, C_1—H +steroidal NH, becoming 1H after deuteration)
					H	8.27	8.38		
					N	6.76	6.95		
XVI	Reflux (1 hr)	76	149–151°	$C_{26}H_{29}BrN_2O_2S$	C	60.81	61.10	244sh (4.276), 277 (4.340)	
					H	5.65	5.78		
					N	5.45	5.70		
XVII	Reflux (3 hr)	93	215–217°	$C_{27}H_{32}N_2O_2S$	C	72.29	72.29	244 (4.233), 280 (3.482)	0.89 (s, 3, C_{18}—CH_3), 3.77 (s, 3, OCH_3), 4.88 (m, 2, $CH_2C_6H_5$), 5.91 (m, 1, benzyl NH, disappearing on deuteration), 6.81 (d, 1, J = 9 Hz, C_2—H), 7.25–7.38 (m, 2, C_1—H + steroidal NH, overlapping with aromatic H), 7.31 (s, 5, aromatic H)
					H	7.19	7.29		
					N	6.25	6.40		

[a] The products were crystallized from benzene–light petroleum except XII and XVII which were crystallized from ethanol–benzene.

cleavage of C_6 and C_7 from the steroidal nucleus to give ion B at m/z 385 and 419, which on losing the thiourea function gave ion C at m/z 256. The thiourea functions in XV and XVII were found to undergo elimination of hydrogen sulfide giving the carbodiimide ion D, at m/z 380 and 414, fission of the isothiocyanate function (14) giving ion E at m/z 299, and cleavage of a butyl or benzylamino function to yield ion F at m/z 341. Ion E in turn, lost an NH function giving ion G at m/z 284, while ion F cleaved sulfur to give the isocyanide ion H at m/z 309.

Table II—Synthesized 2-(2′,4′-Disubstituted thiazolin-3′-yl)-3-methoxyestra-1,3,5(10)-trien-17-ones (XVIII–XXIII) and 4-(2′,4′-Disubstituted thiazolin-3′-yl)-3-methoxyestra-1,3,5(10)-trien-17-ones (XXIV–XXXII)

Compound	Reflux Time	Yield, %	Melting Point[a]	Molecular Formula	Analysis, %[b] C	H	N	UV λ_{max} (log ϵ)[c]
XVIII	30 min	76	183–185° (ethanol–benzene)	$C_{37}H_{38}N_2O_2S \cdot \frac{1}{2} H_2O$	76.15 76.52	6.68 6.97	4.80 5.12	264 (4.614), 300 (4.374)/ 278 (4.633)
XIX	30 min	67	122–124° (aqueous ethanol)	$C_{31}H_{33}ClN_2O_2S$	69.85 69.42	6.19 6.13	5.25 5.35	296 (4.194)/ 288sh (4.599), 264 (4.303)
XX	1.5 hr	74	129–131° (ethanol)	$C_{38}H_{42}N_2O_2S$	77.26 77.07	7.17 7.36	4.74 4.98	266 (4.570), 300sh (4.326)/ 278 (4.589)
XXI	30 min	78	217–219° (benzene–light petr.)	$C_{35}H_{36}N_2O_2S$	76.62 76.41	6.61 6.75	5.11 5.22	295 (4.177)/ 262 (4.253), 290sh (4.156)
XXII	1 hr	85	130–132° (methanol)	$C_{35}H_{35}ClN_2O_2S$	72.10 72.14	6.00 6.26	4.80 5.02	300 (4.252)/ 260sh (4.280), 266 (4.307)
XXIII	30 min	83	124–126° (ethanol)	$C_{41}H_{40}N_2O_2S$	78.82 78.40	6.45 6.69	4.48 4.63	256 (4.596), 296 (4.374)/ 271 (4.619)
XXIV	1 hr	96	138–140° (aqueous methanol)	$C_{31}H_{34}N_2O_2S$	74.67 74.47	6.87 7.22	5.62 5.83	288 (4.149)/ 266 (4.201)
XXV	1 hr	86	155–157° (aqueous ethanol)	$C_{31}H_{33}ClN_2O_2S \cdot \frac{1}{2} H_2O$	68.69 69.18	6.27 6.57	5.17 5.65	266sh (4.272), 290 (4.196)/ 226sh (4.635), 268 (4.404)
XXVI	1 hr	80	145–147° (aqueous ethanol)	$C_{37}H_{38}N_2O_2S$	77.32 77.21	6.67 6.84	4.87 4.96	258 (4.594), 320sh (5.034)/ 270 (4.622)
XXVII	1.5 hr	74	125–127° (aqueous ethanol)	$C_{38}H_{42}N_2O_2S$	77.26 77.52	7.17 7.29	4.74 4.80	258 (4.612), 320sh (5.027)/ 270 (4.632)
XXVIII	2 hr	83	114–116° (aqueous ethanol)	$C_{32}H_{37}ClN_2O_2S$	70.00 69.69	6.74 7.06	5.10 5.24	262 sh (4.287), 289 (4.225)/ 220 (4.659), 263 (4.411)
XXIX	2 hr	63	118–120° (aqueous ethanol)	$C_{32}H_{37}BrN_2O_2S$	64.75 64.84	6.23 6.33	4.72 4.81	231 sh (4.604), 265sh (4.201) 290 (4.104)/ 231 (4.536), 268 (4.225)
XXX	1.5 hr	82	126–128° (aqueous ethanol)	$C_{35}H_{36}N_2O_2S$	76.62 76.67	6.61 6.80	5.11 5.42	288 (4.080)/ 269 (4.212)
XXXI	1.5 hr	79	233–235° (ethanol–benzene)	$C_{41}H_{40}N_2O_2S$	78.82 78.54	6.45 6.52	4.48 5.00	263 (4.100), 300 sh (4.563)/ 282 (4.602)
XXXII	1.5 hr	82	154–156° (aqueous ethanol)	$C_{35}H_{35}BrN_2O_2S$	66.98 66.59	5.58 5.60	4.46 4.31	277 sh (4.632), 289 (4.176)/ 265 (4.376)

[a] With crystallization solvent in parentheses. [b] The first row are calculated values; the second row are the values found. [c] Using ethanol/using ethanol + HCl.

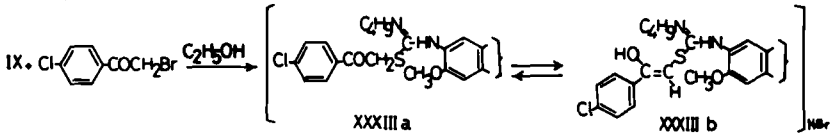
The mass spectrum of 2-(2′-benzylamino-4′-biphenylthiazolin-3′-yl)-3-methoxyestra-1,3,5(10)-trien-17-one (XXIII) showed the molecular ion peak at *m/z* 624 (Scheme IV). In accordance with Pathway 1 (Scheme IV), the fragmentation of the molecule was found to be elimination of an ethylene function from ring B and three hydrogens giving ion A at *m/z* 593. This pathway was confirmed by the appearance of the metastable peak at 563.75. In Pathway 2, the molecule fragmented through elimination of a benzyl function to yield ion B at *m/z* 533, while in Pathway 3 the biphenyl group of the thiazoline moiety was eliminated and ring D removed to give ion C, as the base peak, at *m/z* 373. Ion C, in turn, was found to undergo cleavage through removal of the phenyl group of the benzyl moiety, a thioketene function from the thiazoline ring, and the methoxyl group and then cyclized to ion D at *m/z* 209. The removal of only the phenyl and thioketene moieties from ion C gave the carbodiimide ion F at *m/z* 238, which after cleaving carbon and a methyl radical produced ions G and H at *m/z* 226 and 211, respectively. The spectra of all examined compounds have also shown the different ions corresponding to the reported fragmentation of the steroidal nucleus (15), as well as those of susbstituted thiazoline ring (16–18) (Table III).

Biological Screening—Compounds VIII, X, XVI–XVIII, XX, XXII, XXV, XXIX, and XXX were tested *in vitro* for possible anabolic–catabolic activity by measuring their effect on the activity of bovine pancreatic ribonuclease, as previously reported (7, 19). The results (Table IV) indicated that the steroidal thiazoline derivatives caused a more potent activation of the enzyme than the corresponding steroidal thioureas. In addition, the fact that the thiourea XVI and the thiazolines XXV and XXX caused the highest percentage activation of the enzyme suggested that substitution, especially with the bulky thiazoline moieties, in the 2-position of estrone greatly hinders the binding of the hormonal derivative with the enzyme. The high percentage of enzyme activation caused by the pseudothiourea XXXIII has been attributed to its high polar structure and malleability in spatial arrangement, which allows maximum contact with the enzyme. The thiazoline derivatives XVIII and XXV, having allyl and biphenyl or *p*-chlorophenyl moieties in the heterocyclic ring, as well as XXX, containing the benzyl and phenyl groups, and the steroidal pseudothiourea XXXIII are most likely to possess catabolic activity being the most potent activators of the enzyme. Such a property, effective in suppressing cancer metastasis (20, 21), has recommended such compounds for evaluation for anticancer activity. Compared with these results, the correlative studies from this laboratory indicated that the fusion of a substituted oxazole or oxazoline ring to the 2,3- or 3,4-positions of estrone causes the products to induce a mild or similar percentage of enzyme activation (6, 7).

EXPERIMENTAL[1]

Steroidal Thiourea Derivatives (VIII–XVII)—A solution of the steroidal amines VI and VII (9) (200 mg, 0.66 mmole) and the selected alkyl, aryl, or aralkylisothiocyanate derivative (1.5 *M* equivalent) in ethanol (20 ml) was left at room temperature or heated under reflux as specified in Table I. Ethanol was removed under reduced pressure, and the residue was covered with light petroleum and stored in the refrigerator for 1 hr. The solvent was decanted, and the residue was treated with fresh light petroleum and scratched to deposit the solid. This was filtered, crystallized from the proper solvent, and identified by elemental analysis and IR, UV, and ^{1}H-NMR spectra (Table I). IR (mineral oil): ν 3380–3140 (N—H), 1735–1710 (C=O), 1605–1585 and 1500–1480 (C=C, aromatic), 1535–1520, 1340–1310, 1190–1170, and 950–905 (N—C=S amide I, II, III, and IV bands, respectively), and 1290–1240 and 1080–1050 cm^{-1} (C—O—C). The mass spectrum of XV showed *m/z* (relative abundance %): M^+ at 414(18), 385(10), 384(28), 383(100), 382(15), 381(47), 380(55), 342(22), 341(98), 324(3), 309(10), 300(18), 299(78), 298(6), 285(10), 284(18), 283(5), 256(10), 243(12), 231(10), 230(12), 228(6), 217(17), 216(7), 215(6), 213(5), 212(6), 211(7), 199(7), 198(10), 197(6), 186(10), 185(8), 184(11), 175(8), 174(10), 173(11), 172(13), 171(10), 160(13), 159(10), 158(17), 147(8), 146(15), 145(6), 144(8), 143(10), 142(8), 141(11), 131(12), 130(3), 129(13), 128(17), 116(12), 115(30), 103(12), 102(5), 97(10), 81(8), 79(8), 72(5), 57(25), 55(23), 53(12), and 41(43); the mass spectrum of XVII showed *m/z* (relative abundance %): M^+ at 448(15), 419(10), 418(32), 417(100), 416(13), 415(42), 414(48), 342(4), 341(17), 309(2), 300(10), 299(37), 298(4), 285(3), 284(8), 283(3), 256(3), 243(3), 231(3), 230(3), 228(3), 217(4), 216(3), 215(3), 213(4), 212(4), 211(6), 199(5), 198(6), 197(42), 186(6), 185(5), 184(7), 175(5), 174(6), 173(6), 172(8), 171(6), 165(6), 160(10), 159(5), 158(10), 149(3), 147(4), 146(8), 145(4), 144(6), 143(7), 142(6), 141(72), 131(8), 130(12), 129(10), 128(12), 117(7), 107(6), 106(27), 97(5), 92(18), 91(100), 79(13), 78(7), 77(7), and 41(23).

[1] All melting points are uncorrected. IR spectra were measured as Nujol mulls on a Beckmann 4210 IR Spectrophotometer, and UV spectra for ethanol solution were measured on a Shimadzu double-beam spectrophotometer (Model UV-200S). ^{1}H-NMR and mass spectra were measured on a Perkin-Elmer R32 and an AEI-MS-50, respectively.



Scheme II

2- and 4-(2′,4′-Disubstituted thiazoline-3′-yl)-3-methoxyestra-1,3,5(10)-trien-17-ones (XVIII–XXXII)—A mixture of the thiourea derivatives (VIII, IX, XIII–XV, and XVII) (200 mg) and the molar equivalent of substituted phenacyl bromide in absolute ethanol (10 ml) was heated under reflux for the specified time (Table II). Ethanol was removed under reduced pressure, and the oily residue was dissolved in chloroform (75 ml). The solution was washed with water (3 × 50 ml), dried (anhydrous sodium sulfate), and evaporated to give an oily residue. On scratching with light petroleum, the products deposited in solid form; they were crystallized from the proper solvents and identified by elemental analysis and IR, UV, ^{1}H-NMR, and mass spectra (Tables II and III). IR (mineral oil): ν 1735–1725 (C=O), 1620–1610 (C=N), 1595–1560 and 1500–1490 (C=C, aromatic), and 1265–1235 and 1090–1075 cm^{-1} (C—O—C).

Table III—^{1}H-NMR and Mass Spectral Data of the Steroidal Thiazoline Derivatives

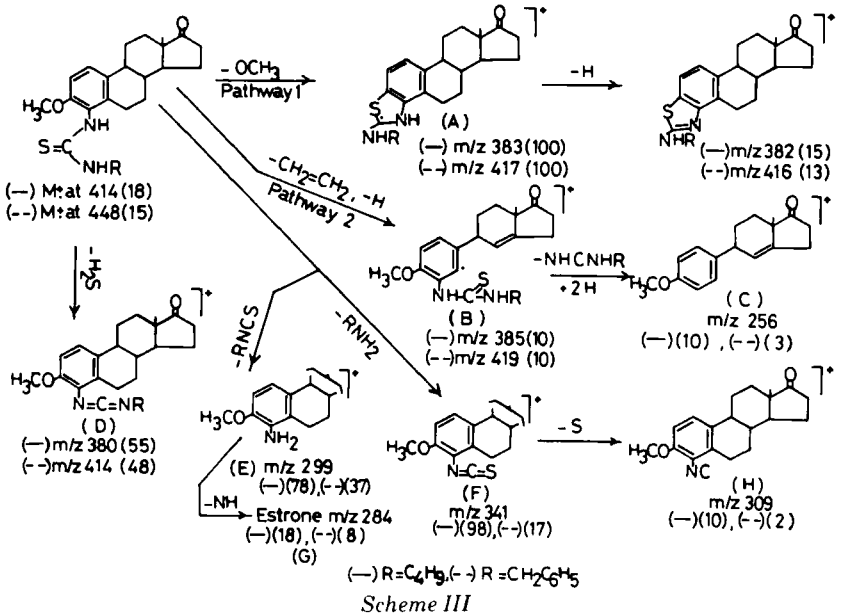
Compound	^{1}H-NMR (δ), ppm	Mass Spectrum, *m/z* (Relative Abundance, %)
XVIII	0.9 (s, distorted, 3, C_{18}—CH_3), 3.81 (s, 3, OCH_3), 4.51 (m, 2, allyl H), 4.94 (m, 1, allyl H), 5.16 (m, 1, allyl H), 5.6–6.2 (group of singlets, 1, allyl H), 5.8 (s, 1, thiazoline H), 6.67 (s, 1, C_4—H), 7.0 (s, 1, C_1—H), 7.3–7.75 (m, 9, aryl H)	
XIX	0.9 (s, 3, C_{18}—CH_3), 3.8 (s, 3, OCH_3), 4.46 (m, 2, allyl H), 4.95 (m, 1, allyl H), 5.2 (m, 1, allyl H), 5.6–6.2 (group of singlets, 1, allyl H), 5.78 (s, 1, thiazoline H), 6.64 (s, 1, C_4—H), 7.0 (s, 1, C_1—H), 7.35 (s, 4, aryl H)	534(45), 533(40), 532(100), 531(13), 520(5), 519(13), 518(10), 517(29), 505(5), 504(15), 503(45), 502(38), 501(100), 494(2), 493(5), 492(6), 491(10), 324(11), 323(33), 322 (9), 298(9), 297(8), 296(4), 236(5), 225(4), 224(5), 223(8), 211(5), 210(6), 209(6), 199(4), 198(4), 197(5), 196(8), 194(2), 186(4), 185(5), 184(5), 173(5), 172(5), 171(5), 170(6), 169(6), 168(15), 160(11), 159(63), 158(4), 155(5), 143(4), 141(6), 140(3), 138(5), 136(5), 134(8), 133(5), 130(4), 129(8), 128(8), 116(5), 115(10), 103(4), 97(4), 55(11), 41(50).
XX	0.84 (t, 3, J = 7 Hz, CH_2—CH_3), 0.9 (s, 3, C_{18}—CH_3), 3.8 (s, 3, OCH_3), 3.88 (m, 2, N—CH_2—), 5.75 (s, 1, thiazoline H), 6.65 (s, 1, C_4—H), 7.0 (s, 1, C_1—H), 7.25–7.75 (group of multiplets, 9, aryl h)	
XXI	0.9 (s, 3, C_{18}—CH_3), 3.76 (s, 3, OCH_3), 5.1 (s, 2, —$CH_2C_6H_5$), 5.73 (s, 1, thiazoline H), 6.64 (s, 1, C_4—H), 6.98 (s, 1, C_1—H), 7.04–7.40 (group of multiplets, 10, aryl H)	
XXII	0.9 (s, 3, C_{18}—CH_3), 3.75 (s, 3, OCH_3), 5.08 (s, 2, —$CH_2C_6H_5$), 5.73 (s, 1, thiazoline H), 6.64 (s, 1, C_4—H), 6.96 (s, 1, C_1—H), 7.03–7.33 (group of multiplets, 9, aryl H)	
XXIII	0.9 (s, 3, C_{18}—CH_3), 3.78 (s, 3, OCH_3), 5.15 (s, 2, —$CH_2C_6H_5$), 5.8 (s, 1, thiazoline H), 6.65 (s, 1, C_4—H), 6.99 (s, 1, C_1—H), 7.19 (s, 5, phenyl H), 7.22–7.66 (group of multiplets, 9, aryl H)	625(19), 624(40), 595(4), 594(10), 593(22), 534(10), 533(14), 395(2), 374(29), 373(100), 371(4), 370(6), 369(23), 297(5), 296(3), 238(6), 226(2), 225(3), 224(13), 211(9), 210(24), 209(6), 208(3), 199(2), 198(2), 197(6), 184(4), 181(8), 180(4), 179(6), 178(13), 172(2), 171(2), 167(4), 161(8), 159(2), 158(2), 153(4), 141(3), 129(5), 128(4), 116(3), 115(6), 107(3), 106(5), 105(5), 97(3), 92(14), 91(67), 77(4), 41(9).
XXIV	0.9 (s, 3, C_{18}—CH_3), 3.8 (s, 3, OCH_3), 4.5 (m, 2, allyl H), 4.99 (m, 1, allyl H), 5.21 (m, 1, allyl H), 5.76 (s, 1, thiazoline H), 5.72–6.06 (group of singlets, 1, allyl H), 6.81 (d, 1, J = 9 Hz, C_2—H), 7.05 (d, 1, J = 9 Hz, C_1—H), 7.45 (s, 5, aryl H)	
XXVI	0.9 (s, 3, C_{18}—CH_3), 3.82 (s, 3, OCH_3), 4.57 (m, 2, allyl H), 5.06 (m, 1, allyl H), 5.22 (m, 1, allyl H), 5.81 (s, 1, thiazoline H), 5.8–6.35 (group of singlets, 1, allyl H), 6.82 (d, 1, J = 9 Hz, C_2—H), 7.07 (d, 1, J = 9 Hz, C_1—H), 7.4–7.82 (m, 9, aryl H)	
XXVII	0.73 (t, 3, J = 7 Hz, CH_2—CH_3), 0.91 (s, 3, C_{18}—CH_3), 3.82 (s, 3, OCH_3), 3.98 (t, 2, J = 7 Hz, —N—CH_2—), 5.77 (s, 1, thiazoline H), 6.85 (d, 1, J = 9 Hz, C_2—H), 7.08 (d, 1, J = 9 Hz, C_1—H), 7.39–7.82 (m, 9, aryl H)	
XXVIII	0.8 (t, 3, J = 7 Hz, CH_2CH_3), 0.91 (s, 3, C_{18}—CH_3), 3.82 (s, 3, OCH_3), 3.91 (t, 2, J = 7 Hz, N—CH_2—), 5.75 (s, 1, thiazoline H), 6.85 (d, 1, J = 9 Hz, C_2—H), 7.91 (d, 1, J = 9 Hz, C_1—H), 7.45 (m, 4, aryl H)	550(46), 549(39), 548(100), 547(8), 535(2), 533(6), 521(3), 520(3), 519(8), 518(5), 517(13), 515(6), 495(8), 495(24), 493(21), 492(55), 491(4), 480(2), 479(4), 478(4), 477(12), 475(9), 464(2), 463(5), 462(4), 461(10), 339(5), 338(5), 322(6), 321(6), 309(8), 298(9), 297(33), 296(6), 290(4), 282(9), 252(14), 250(18), 211(6), 210(12), 209(6), 198(6), 196(18), 194(11), 186(4), 185(4), 184(6), 174(4), 173(4), 172(5), 171(6), 170(4), 169(9), 168(8), 160(4), 159(3), 158(5), 157(4), 155(4), 143(4), 141(4), 140(8), 138(24), 136(5), 134(12), 133(5), 129(6), 128(5), 116(3), 115(7), 103(3), 97(5), 57(12), 55(14), 41(52).
XXIX	0.74 (t, 3, J = 7 Hz, CH_2CH_3), 0.91 (s, 3, C_{18}—CH_3), 3.82 (s, 3, OCH_3), 3.91 (t, 2, J = 7 Hz, —N—CH_2—), 5.72 (s, 1, thiazoline H), 6.85 (d, 1, J = 9 Hz, C_2—H), 7.08 (d, 1, J = 9 Hz, C_1—H), 7.31 (d, 2, J = 9 Hz, aryl H), 7.65 (d, 2, J = 9 Hz, aryl H)	
XXXI	0.9 (s, 3, C_{18}—CH_3), 3.81 (s, 3, OCH_3), 5.21 (s, 2, —$CH_2C_6H_5$), 5.83 (s, 1, thiazoline H), 6.81 (d, 1, J = 9 Hz, C_2—H), 7.03 (d, 1, J = 9, Hz, C_1—H), 7.29 (s, 5, $CH_2C_6H_5$), 7.2–7.72 (m, 9, biphenyl H)	625(18), 624(36), 596(4), 595(14), 594(39), 593(84), 535(13), 533(3), 395(20), 374(3), 373(8), 370(2), 369(5), 297(2), 238(11), 226(13), 225(3), 224(3), 211(8), 210(20), 209(6), 208(3), 198(2), 197(4), 184(3), 181(3), 180(4), 179(5), 178(16), 172(2), 167(4), 153(3), 141(3), 129(3), 128(9), 116(2), 115(5), 107(1), 106(1), 97(2), 92(13), 91(100), 77(3), 41(6).

Table IV—In Vitro Effects of the Synthesized Steroidal Thiourea and Thiazoline Derivatives on the Activity of Bovine Pancreatic Ribonuclease

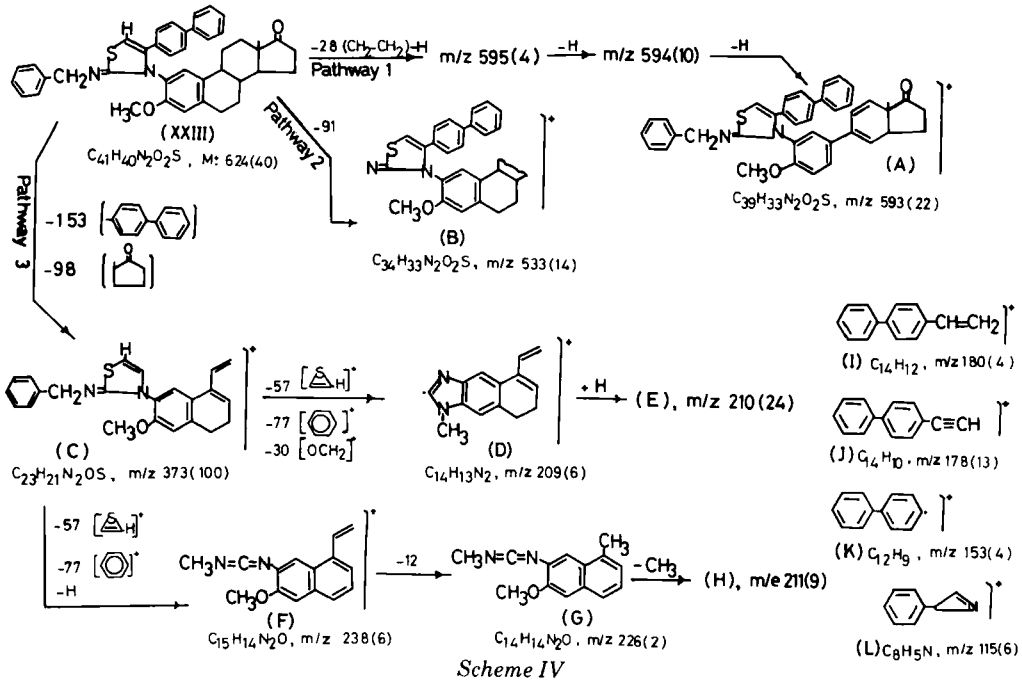
Compound		Molar Concentration of the Products (M)			
		10^{-6}	10^{-7}	10^{-8}	10^{-9}
I	Mean ± SE[a]	219.6 ± 10.03	225.4 ± 20.04	231.2 ± 14.4	230 ± 17.02
	% Activation	12.46	15.61	18.57	17.98
	p Value	>0.05	>0.05	>0.05	>0.05
	Control		(200.2 ± 14.2)		
VIII	Mean ± SE	278.2 ± 8.12	298.6 ± 26.8	274.6 ± 25.1	286.3 ± 18.6
	% Activation	39.20	49.22	37.50	43.12
	p Value	<0.001	<0.001	<0.05	<0.001
X	Mean ± SE	276.2 ± 25.1	256.5 ± 12.2	254.6 ± 18.2	282.4 ± 13.2
	% Activation	37.98	28.12	27.21	41.26
	p Value	<0.01	<0.01	<0.01	<0.001
XVI	Mean ± SE	259.2 ± 14.4	360.6 ± 10.6	438.3 ± 15.4	464.1 ± 24.8
	% Activation	29.5	80.2	119.2	132.3
	p Value	<0.01	<0.001	<0.001	<0.001
XVII	Mean ± SE	202.2 ± 19.2	223.6 ± 12.8	205.3 ± 12.3	234.6 ± 12.9
	% Activation	0.99	11.68	2.55	17.18
	p Value	>0.05	>0.05	>0.05	>0.05
	Control		(195 ± 15.2)		
XVIII	Mean ± SE	384 ± 18.2	322 ± 10.4	377 ± 21.3	375 ± 20.3
	% Activation	96.92	95.90	93.33	92.30
	p Value	<0.001	<0.001	<0.001	<0.001
XX	Mean ± SE	280 ± 11.5	275 ± 19.4	268 ± 13.2	325 ± 19.6
	% Activation	44.06	41.10	37.20	66.66
	p Value	>0.05	>0.05	>0.05	>0.01
XXII	Mean ± SE	246 ± 22.32	260 ± 28.3	295 ± 21.2	323 ± 16.6
	% Activation	26.57	33.39	51.77	65.99
	p Value	>0.05	<0.05	<0.01	<0.01
XXV	Mean ± SE	362 ± 25.5	402 ± 14.6	379 ± 16.2	467 ± 13.2
	% Activation	85.64	106.15	94.16	139.49
	p Value	<0.001	<0.001	<0.001	<0.001
XXIX	Mean ± SE	206 ± 22.2	196 ± 11.3	206 ± 20.6	198 ± 13.5
	% Activation	5.64	0.51	5.64	1.54
	p Value	>0.05	>0.05	>0.05	>0.05
XXX	Mean ± SE	314 ± 13.2	405 ± 17.3	453 ± 16.2	512 ± 22.3
	% Activation	61.03	107.69	132.31	162.56
	p Value	<0.001	<0.001	<0.001	<0.001
XXXIII	Mean ± SE	450 ± 26.1	528 ± 14.3	246 ± 26.3	227 ± 24.1
	% Activation	130.77	170.77	26.15	16.41
	p Value	<0.001	<0.001	>0.05	>0.05

[a] The ribonuclease activity is expressed in units as the mean value ± standard error.

***N*-(3-Methoxy-17-oxoestra-1,3,5(10)-trien-2-yl)-*S*-(*p*-chlorophenacyl)-*N'*-butyl-pseudothiourea Hydrobromide (XXXIII)**—A mixture of equimolar amounts of *N*-butyl-*N'*-(3-methoxy-17-oxoestra-1,3,5(10)-trien-2-yl)thiourea (IX) (200 mg) and 4-chlorophenacyl bromide in absolute ethanol (10 ml) was heated under reflux for 2 hr. The ethanol was evaporated to dryness, and the oily residue was dissolved in chloroform (75 ml) and washed with water (3 × 50 ml). The chloroform layer was dried (anhydrous sodium sulfate) and evaporated to dryness; the product was scratched with light petroleum to give a solid which was filtered and dried. Crystallization from benzene–light petroleum gave 160 mg (60% yield) of a yellowish amorphous solid, mp 208–210° (dec). IR (mineral oil): ν 3400 (N—H broad), 1735 (C=O), 1620 (C=N), 1590 and 1500 (C=C, aromatic), and 1255 and 1090 cm^{-1} (C—O—C); UV (ethanol): λ_{max} (log ϵ) 300 (4.209); UV (ethanol–HCl): λ_{max} (log ϵ) 228 sh (4.585), 266 (4.272), and 295 sh (4.107); ^{1}H-NMR ($CDCl_3$): δ 0.85 (t, 3, J = 6 Hz, butyl-CH_3, overlapping with the singlet of C_{18}—CH_3), 0.88 (s,



Scheme III



Scheme IV

3, C_{18}—CH_3), 3.79 (s, 3, OCH_3), 4.61 (t, 2, J = 6 Hz, —CH_2N—), 6.56 (s, 1, S—C*H*=C—OH), 6.7 (s, 1, C_4—H), 7.28 (s, 1, C_1—H), and 7.47 (m, 5, 4 aromatic protons + steroidal N—H).

Anal. —Calc. for $C_{32}H_{40}BrClN_2O_2S \cdot \frac{1}{2} H_2O$: C, 59.03; H, 6.30; N, 4.30. Found: C, 59.19; H, 6.88; N, 4.67.

***In Vitro* Anabolic–Catabolic Activities**—Four sets of solutions and media (7) were used in the evaluation procedures. After mixing the components of each set, the tubes were incubated at 37° for 15 min and then treated with 4 ml of ethanol–glacial acetic acid (15:1, v/v) to terminate the reaction. After storage for 1 hr in the refrigerator, the tubes were centrifuged for 15 min and the clear supernatants were measured spectrophotometrically at 260 nm. The results of the effect of VIII, X, XVI–XVIII, XX, XXII, XXV–XXIX, XXX, and XXXIII on the activity of bovine pancreatic ribonuclease are shown in Table IV.

REFERENCES

(1) A.-Mohsen M. E. Omar, S. M. El-Khawass, A. B. Makar, N. M. Bakry, and T. T. Daabees, *Pharmazie*, **33**, 577 (1978).

(2) A.-Mohsen M. E. Omar and F. A. Ashour, *Pharmazie*, **33**, 747 (1979).

(3) S. M. El-Khawass, A.-Mohsen M. E. Omar, T. T. Daabees, and F. M. Sharaby, *Pharmazie*, **35**, 143 (1980).

(4) A.-Mohsen M. E. Omar, A. M. Farghaly, A. A. B. Hazzaa, and N. H. Eshba, *Pharmazie*, **35**, 809 (1980).

(5) El-Sebaii A. Ibrahim, A.-Mohsen M. E. Omar, M. A. Khalil, A. B. Makar, and T. T. Daabees, *Pharmazie*, **35**, 810 (1980).

(6) El-Sebaii A. Ibrahim, A.-Mohsen M. E. Omar, N. S. Habib, and Omaima M. AboulWafa, *J. Heterocycl. Chem.*, **19**, 761 (1982).

(7) A.-Mohsen M. E. Omar and Omaima M. AboulWafa, *J. Pharm. Sci.*, **71**, 983 (1982).

(8) R. A. Pickering and H. Werbin, *J. Am. Chem. Soc.*, **80**, 680 (1958).

(9) S. Kraychy, *J. Am. Chem. Soc.*, **81**, 1702 (1959).

(10) A. J. Tomson and J. P. Horwitz, *J. Org. Chem.*, **24**, 2056 (1959).

(11) A.-Mohsen M. E. Omar, N. S. Habib, and Omaima M. AboulWafa, *Pharmazie*, **32**, 758 (1977).

(12) A.-Mohsen M. E. Omar, S. A. Shams-El-Din, A. A. Ghobashy, and M. A. Khalil, *Eur. J. Med. Chem.*, **16**, 77 (1981).

(13) E. R. Clark, A.-Mohsen M. E. Omar, and G. Prestwich, *J. Med. Chem.*, **20**, 1096 (1977).

(14) A.-Mohsen M. E. Omar, A. M. Farghaly, A. A. B. Hazzaa, N. H. Eshba, F. M. Sharaby, and T. T. Daabees, *J. Pharm. Sci.*, **70**, 1075 (1981).

(15) C. Djerassi, J. M. Wilson, H. Budzikiewicz, and J. W. Chamberlain, *J. Am. Chem. Soc.*, **84**, 4544 (1962).

(16) H. Ogura, S. Sugimoto, and T. Itoh, *Org. Mass Spectrom.*, **3**, 1341 (1970).

(17) G. M. Clarke, R. Grigg, and D. H. Williams, *J. Chem. Soc. (B)*, **1966**, 339.

(18) M. J. Rix and B. R. Webster, *Org. Mass Spectrom.*, **5**, 311 (1971).

(19) S. M. El-Sewedy, E. A. El-Basiouni, and S. T. Assar, *Biochem. Pharm.*, **27**, 1831 (1978).

(20) A. Graffi and W. Arnold, *Acta Biol. Med. Ger.*, **30**, 15 (1973).

(21) Taik Koo Yun, XI International Cancer Congress, Florence, 1974, Panel 15.

ACKNOWLEDGMENTS

Presented in part at the Symposium on Chemistry in Developing Countries, New York, N.Y., August 1981.

Supported in part by Pharco Pharmaceuticals, Cairo, Egypt. The authors thank Shering AG, Berlin, West Germany, for the donation of estrone. Thanks are also due to Dr. E. Dorme and Mrs. Cabaret, Université de Paris-sud, Orsay, and Dr. D. C. Das, Institut de Chimie des Substances Naturelles, Gif-Sur-Yvette, France, for the microanalytical, ^{1}H-NMR, and mass spectral data.