with abnormal electroencephalographic changes. The pathogenic mechanism involved remains unknown, however, and it is hoped that this report will stimulate research in this area.

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Potential CNS Antitumor Agents—Phenothiazines II: Fluphenazine Analogs

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Abstract □ Fluphenazine was found to possess moderate, reproducible activity against the intraperitoneal L-1210 and P-388 leukemia murine tumor models. Seven ether derivatives of fluphenazine and eight compounds in which the terminal side-chain hydroxyl group was replaced by an amine function were prepared and evaluated in the intraperitoneal L-1210, P-388, and B16 melanoma systems as well as the intracerebral L-1210 and ependymoblastoma brain tumor models. While no substantial intracerebral activity was observed, seven derivatives possessed reproducible activity in the intraperitoneal L-1210 or P-388 system. Several gave T/C values of 150%. No B16 melanoma activity was observed. These compounds were also tested for their cytotoxic properties in culture against L-1210, P-388, and KB cells. The amine isosteres, while possessing little *in vivo* activity, were the most cytotoxic of the compounds prepared, with several having ED_{50} values <1 μg/ml.

Keyphrases □ Fluphenazine and various analogs—synthesized, CNS antitumor and cytotoxic activity evaluated □ Phenothiazines, various—synthesized, CNS antitumor and cytotoxic activity evaluated □ Antitumor activity, CNS—fluphenazine and various analogs evaluated □ Cytotoxic activity—fluphenazine and various analogs evaluated □ Structure-activity relationships—fluphenazine and various analogs evaluated for CNS antitumor and cytotoxic activity

Brain tumors and other cancers of the central nervous system (CNS) continue to constitute a difficult clinical problem. Approximately 8500 patients per year die from brain tumors. CNS cancer is the second most common type in children under 15 years old (1). Secondary CNS tumors are encountered among leukemia patients (2), who are often treated prophylactically with irradiation of the CNS or with intrathecal methotrexate administration (3, 4).

Drugs able to enter the CNS are obvious candidates as potential CNS antitumor agents. Psychotropic drugs in general (5–8) and phenothiazine derivatives in particular (5–20) have been investigated for their antitumor properties. The possibility that phenothiazine derivatives may have had a favorable effect on human carcinomas has been discussed (19).

Although some activity has been observed with phenothiazines in animal tumor models when the tumor was intraperitoneally or subcutaneously implanted, no CNS antitumor activity was found when an intracerebral tumor model was studied using phenothiazine nitrogen mustard derivatives (20). At the time the phenothiazine mustard investigation (20) was initiated, a second, parallel, attempt to produce a CNS antitumor agent based on a nonalkylating phenothiazine system was started. This work was based on the intraperitoneal murine leukemia activity observed during the initial testing of trifluoperazine (I), fluphenazine (II), and the hydroxyethyl ether of fluphenazine (III). Compounds I and II are known to penetrate the blood–brain barrier and to have significant CNS activity in humans. It appeared that if a compound of this type could be discovered that had high intraperitoneal

Table I—Physical and Chemical Data

Compound	Melting Point	Yield, %	Formula	Number of Hydrochlorides	Analysis, %		
						Calc.	Found
V	73.5–74.5°	55	$C_{16}H_{13}ClF_3NS$	0	C	56.06	56.09
					H	3.82	3.78
					Cl	10.34	10.39
					N	4.09	4.08
					S	9.35	9.51
VII	194–197°	85	$C_{24}H_{32}Cl_2F_3N_3OS$	2	C	53.53	53.14
					H	5.99	6.14
					Cl	13.17	12.71
					N	7.80	7.69
					S	5.95	5.97
VIII	192–195°	63	$C_{26}H_{36}Cl_2F_3N_3OS$	2	C	55.12	55.47
					H	6.41	6.09
					Cl	12.52	12.14
					N	7.42	7.28
					S	5.66	5.95
IX	171–173°	58	$C_{24}H_{31}Cl_3F_3N_3OS \cdot \frac{1}{2}H_2O$	2	C	49.53	49.38
					H	5.54	5.57
					Cl	18.28	18.30
					N	7.22	7.16
					S	5.51	5.43
X	154–155°	67	$C_{25}H_{34}Cl_2F_3N_3O_2S \cdot H_2O$	2	C	51.19	50.89
					H	6.19	6.01
					Cl	12.09	11.92
					N	7.16	7.02
					S	5.47	5.50
XI	188–191°	63	$C_{25}H_{32}Cl_2F_3N_3OS \cdot \frac{1}{2}H_2O$	2	C	53.66	53.27
					H	5.94	5.97
					Cl	12.67	12.75
					N	7.51	7.30
					S	5.73	5.86
XII	181–183°	77	$C_{27}H_{38}Cl_2F_3N_3OS$	2	C	55.89	55.82
					H	6.55	6.81
					Cl	12.22	12.27
					N	7.24	7.00
					S	5.53	5.31
XIII	192–195°	77	$C_{28}H_{40}Cl_2F_3N_3OS$	2	C	56.59	56.34
					H	6.73	6.85
					Cl	11.93	12.21
					N	7.07	6.90
					S	5.40	5.28
XIV	205–207°	84	$C_{28}H_{32}Cl_2F_3N_3OS$	2	C	57.36	57.13
					H	5.46	5.66
					Cl	12.09	11.82
					N	7.16	6.95
					S	5.47	5.41
XV	181–185°	63	$C_{24}H_{34}Cl_3F_3N_4OS \cdot \frac{1}{2}H_2O$	3	C	47.41	47.16
					H	5.80	5.88
					Cl	17.50	17.38
					N	9.22	9.03
					S	5.27	5.43
XVI	217–219°	69	$C_{25}H_{36}Cl_3F_3N_4OS$	3	C	49.71	49.39
					H	6.01	6.31
					Cl	17.61	17.60
					N	9.28	9.19
					S	5.31	5.23
XVII	207–210°	61	$C_{25}H_{34}Cl_3F_3N_4S \cdot \frac{1}{2}H_2O$	3	C	50.42	50.05
					H	5.93	5.67
					Cl	17.88	17.78
					N	9.42	9.12
					S	5.39	5.57
XVIII	204–206°	74	$C_{28}H_{38}Cl_3F_3N_4S$	3	C	53.72	53.83
					H	6.12	6.46
					Cl	16.99	16.80
					N	8.95	8.82
					S	5.12	5.14
XIX	253–256°	73	$C_{27}H_{38}Cl_3F_3N_4S$	3	C	52.81	52.53
					H	6.24	5.98
					Cl	17.32	16.88
					N	9.12	9.20
					S	5.22	5.13
XX	255–257°	63	$C_{26}H_{36}Cl_3F_3N_4OS \cdot \frac{1}{2}H_2O$	3	C	49.96	49.98
					H	5.97	5.93
					Cl	17.02	17.17
					N	8.96	8.84
					S	5.13	5.50
XXI	202–205°	38	$C_{24}H_{35}Cl_3F_3N_5S \cdot 2H_2O$	3	C	46.12	46.43
					H	6.29	5.91
					Cl	17.02	17.20
					N	11.21	11.10
					S	5.13	5.32

Table I— *(Continued)*

Compound	Melting Point	Yield, %	Formula	Number of Hydrochlorides	Analysis, %	Calc.	Found
XXII	135–137°	47	$C_{34}H_{37}F_3N_4O_3S_2$	0	C	60.87	60.92
					H	5.56	5.41
					N	8.35	8.04
					S	9.56	9.29
XXIII	238–240°	13	$C_{24}H_{29}ClF_3N_3OS \cdot H_2O$	0	C	55.65	56.06
					H	5.99	5.91
					Cl	6.86	7.02
					N	8.12	8.33
					S	6.18	6.18

antitumor activity (*e.g.*, T/C 200%)[1], it might also possess a degree of CNS antitumor activity. Therefore, an investigation was started to explore the effect on the antitumor activity of altering the hydroxyethyl side chain of II.

CHEMISTRY

Since III had antitumor activity equal to or better than the parent compound, the effect of modifying the terminal hydroxyl group in the II side chain was investigated. To provide as much nonlinearity as possible among the physicochemical parameters, σ^* was plotted against π (21), and these data were used as much as possible for the selection of candidate R groups (Scheme I). An attempt also was made to provide some steric differences among the various R groups. The effect of replacing the terminal hydroxyl group by an amine function (XV–XXII) also was investigated.

The synthetic procedure used is shown in Scheme I. Compound IV was alkylated in the 10-position to give V, using the general procedure employed by Jackson and Shirley (13) to produce the corresponding β-chloroethyl compound. The reaction of V with piperazineethanol provided II, which was chlorinated to produce the key intermediate (VI). Compound VI then was reacted with the appropriate sodium alkoxide or amine to give the desired analogs. Physical and chemical properties of these materials are listed in Table I.

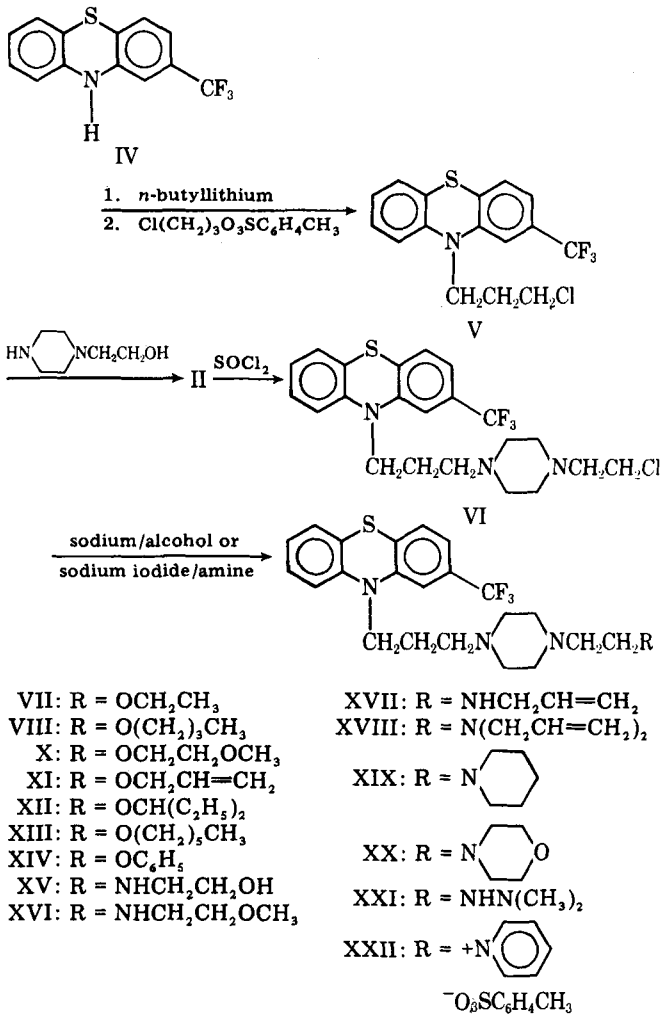
When III was reacted with thionyl chloride (Scheme II), an intramolecular alkylation product (XXIII) was isolated in addition to the desired product (IX). If the reaction mixture was chromatographed on alumina, XXIII was produced exclusively. Chromatography under less basic conditions (silica gel) retarded the intramolecular reaction and gave IX and XXIII in yields of 58 and 13%, respectively.

The NMR spectra of these phenothiazines as free bases in deuterochloroform were similar to the spectrum of the parent compound (II), which had the following resonance characteristics: aromatic protons as a multiplet at δ 7.0 ppm and methylene groups attached to the phenothiazine nitrogen, to the hydroxyl group, and at the middle of the propyl chain at δ 3.9 (t), 3.6 (t), and 1.9 (t) ppm, respectively; the methylene groups in and attached to the piperazine ring gave a broad singlet or partially resolved triplet at δ 2.4 ppm while the hydroxyl proton gave a singlet at δ 2.9 ppm. These data are in agreement with those reported earlier for II (22). The major differences in the spectra of XXII and XXIII were the resonance frequencies of the methylene groups attached to the quaternary nitrogen atoms. They occurred at δ 5.1 and 3.8 ppm for these two compounds, respectively.

The elemental analytical values for several compounds (Table I) indicated that they were hemihydrates. While this is not uncommon for derivatives of this type (23), the mass spectra of two such compounds (XV and XVII) were run to confirm their structures. Compound XVII gave a molecular ion at *m/e* 476 while the spectrum of XV showed a peak at *m/e* 462 corresponding to M − H_2O. Both compounds gave strong peaks

I: R = CH_3
II: R = CH_2CH_2OH
III: R = $CH_2CH_2OCH_2CH_2OH$

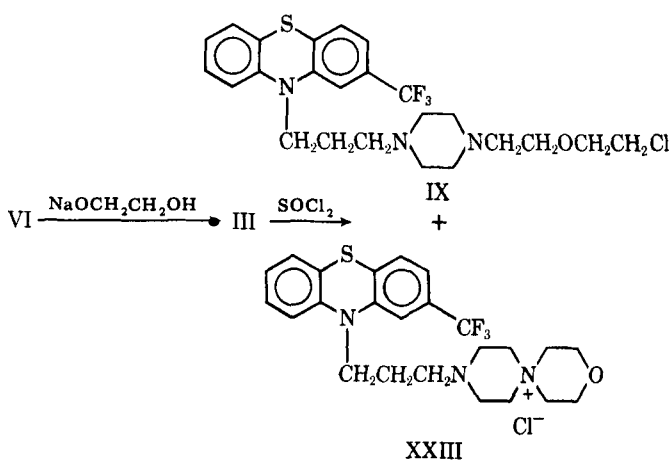
[1] T/C = (treated survival ÷ control survival) × 100%.



VII: R = OCH_2CH_3
VIII: R = $O(CH_2)_3CH_3$
X: R = $OCH_2CH_2OCH_3$
XI: R = $OCH_2CH{=}CH_2$
XII: R = $OCH(C_2H_5)_2$
XIII: R = $O(CH_2)_5CH_3$
XIV: R = OC_6H_5
XV: R = $NHCH_2CH_2OH$
XVI: R = $NHCH_2CH_2OCH_3$

XVII: R = $NHCH_2CH{=}CH_2$
XVIII: R = $N(CH_2CH{=}CH_2)_2$
XIX: R = N⬡
XX: R = N⬡O
XXI: R = $NHN(CH_3)_2$
XXII: R = $+N$⬡ $^-O_3SC_6H_4CH_3$

Scheme I



Scheme II

Table II—Antitumor Activity[a]

Compound	L-1210 Lymphoid Leukemia[b] OD[d]	T/C	P-388 Lymphocytic Leukemia[b] OD	T/C	Ependymoblastoma[c] OD	T/C
I	66	142	100	150	75	115
	37	137	100	163	75	110
II	50	164	25	148	30	110
	25	130	25	145	15	126
III	50	150	25	150	50	106
	120	150	25	126		
V	100	118	200	104		
VI	25	110	25	115		
VII	35	136	12.5	116	37.5	97
	50	132	12.5	138		
VIII	50	122	50	153		
	25	116	50	129		
IX	25	122	25	141		
			50	134		
X	50	151	6.2	161		
	50	138	25	127	37.5	125
XI	35	148	20	144		
	35	129	5	125	50	130
XII	6.2	112	40	131		
	12.5	97	20	109		
XIII	12.5	106	12.5	130		
			12.5	129		
XIV	12.5	126	25	122		
	12.5	104	25	124		
XV	6.2	104	12.5	135		
			12.5	110		
XVI	6.2	110	80	120		
	50	108	40	113		
XVII	6.2	106	50	144	37.5	92
			50	116	50	130
XVIII	25	112	25	112		
			25	111		
XIX	25	96	12.5	118		
XX	25	116	50	112		
			50	104		
XXI	25	104				
XXII	12.5	101	20	126		
			20	122		
XXIII	12.5	114				

[a] Protocols and test systems were described in Ref. 24. The results from two separate experiments are given when available. [b] Day 1, 5, and 9 treatment schedule. Intraperitoneal treatment of intraperitoneal tumor. [c] QD 1–5 treatment schedule. Intraperitoneal treatment of intracerebral tumor. [d] Optimum dose (milligrams per kilogram per injection).

at *m/e* 406 corresponding to M − CH_2NHR. No evidence for the presence of sulfoxide was observed in either sample.

BIOLOGY

Tumor Models Employed—Compounds were tested according to standard National Cancer Institute (NCI) protocols (24). The every 4th day (Q4D-1,5,9) treatment schedule was determined to be superior to the chronic (QD 1–9) or Day 1 only schedule for these compounds in the L-1210 and P-388 tumor systems. Intraperitoneal treatment of intraperitoneally implanted tumor was employed with these two tumor models (Table II).

The compounds with reproducible intraperitoneal L-1210 activity (I–III, VII, X, and XI) were tested in the intracerebral L-1210 and intracerebral ependymoblastoma tumor models (intracerebral tumor implantation and intraperitoneal drug administration). The criterion for statistically significant activity has been established (24) as a T/C value of ≥ 125% in these tumor systems.

Because chlorpromazine had been reported to have significant activity in the B16 melanocarcinoma tumor system (14), all compounds except IV–VI were tested against the intraperitoneal B16 tumor. No significant activity (T/C ≥ 140%) was observed for any compound.

In vitro cytotoxicity data were also obtained using the L-1210, P-388, and KB cell lines (24) (Table III).

***In Vivo* Studies**—Compounds I–III had reproducible, moderate antitumor activity against the intraperitoneal L-1210 and the P-388 tumor systems (Table II). There was not much difference in the activity of the three compounds, each producing T/C values of about 150% (*i.e.*, an increase in lifespan of 50%). Replacing the terminal hydroxyl group of III by a proton gave VII, which retained activity in both systems but at a somewhat lower level. Replacement by a methoxyl group gave X, in which equivalent activity was found; and replacement by an olefin methylene group gave XI, which was approximately as active as III in both tumor systems. Replacement of the hydroxyl group in III by a butyl group (XIII) abolished the L-1210 activity. This compound, however, retained marginal P-388 activity. Nonreproducible P-388 and L-1210 activity was obtained with the 3-pentyl (XII) and phenyl (XIV) ethers of fluphenazine.

Little *in vivo* activity was observed among the amino derivatives. Some marginal P-388 activity was observed with XV (the nitrogen analog of III), the vinyl compound XVII, and the *unsym*-dimethylhydrazine analog XXII.

Because of their activity in one or both intraperitoneal tumor systems, seven fluphenazine analogs were tested in the murine L-1210 and ependymoblastoma (EM) brain tumor models. No activity was seen in the intracerebral L-1210 tests. Although statistically significant activity (T/C ≥ 125%) (24) was observed with II, X, XI, and XVII, this activity was so marginal that for practical purposes it can be concluded that none of the L-1210 intraperitoneal active compounds investigated possesses antitumor activity in either of the two intracerebral model systems studied.

***In Vitro* Studies**—While the simple phenothiazines IV and V are essentially inactive in the three cell culture systems studied (L-1210, P-388, and KB), many II analogs prepared possessed substantial cytotoxicity. While II was moderately cytotoxic, I, III, X, XX, and XXI appeared to be about eight times as cytotoxic as II in the L-1210 and P-388 systems. Most active of all were the nitrogen isosteres XV–XVII with ED_{50} values <1 μg/ml (Table III).

Table III—Cell Culture Activity (Micrograms per Milliliter)[a]

Compound	L-1210	P-388	KB
I	6	6	18
II	49	40	13
III	6	5	11
IV		>100	>100
V	>100	>100	>100
VI		17	7
VII	5	49	18
VIII	56	40	>100
IX	36	7	21
X	6	4	8
XI	25	5	7
XII	>100	54	59
XIII	>100	88	>100
XIV	69	52	>100
XV	0.6	0.4	2
XVI	4	0.5	2.5
XVII	0.9	0.5	6
XVIII	54	44	>100
XIX	2.3	0.39	2.9
XX	5	4	2.5
XXI	6	5	23
XXII	58	43	8
XXIII	59	51	51

[a] Concentration required to inhibit cell growth to 50% that of the control.

For the oxygen analogs prepared (I–III and VII–XIV), *in vitro* cytotoxicity tended to parallel *in vivo* activity. Compounds I, III, VII, X, and XI possessed *in vivo* activity in both the L-1210 and P-388 systems. These compounds also had *in vitro* ED_{50} activities in the 5–20-μg/ml range. Fluphenazine (II), which had substantial intraperitoneal L-1210 and P-388 activity, also was active *in vitro* but at a higher ED_{50} value (40 μg/ml). While VII, the ethyl ether of II, was relatively toxic, the butyl ether (VIII) was less so and the hexyl ether (XIII) was essentially noncytotoxic, suggesting a possible dependence on the lipophilicity of these compounds. It is not possible to determine *in vivo versus in vitro* relationships for the nitrogen analogs because of the generally poor *in vivo* activity of these compounds. These materials, however, were the most cytotoxic compounds prepared.

DISCUSSION

Phenothiazines of the type described here clearly have reproducible, moderate *in vivo* antitumor activity in the intraperitoneal L-1210 and P-388 tumor models and are cytotoxic in cell culture, but the possible utility of the activity observed is presently unclear. CNS agents in general (25) and phenothiazines, such as chlorpromazine, in particular (25, 26) can reduce body temperature (hypothermia) in mice. The apparent an-

titumor activity of reserpine has been attributed to hypothermic effects (27). However, the compounds described in the present study are definitely cytotoxic.

Moreover, materials of this type are known to be surface-active agents (9, 28) capable of modifying cell surface characteristics. They produce numerous other physiological effects (9, 29). The similarity in activity of the best compounds in this series could conceivably be related to metabolic similarities (30). More needs to be known about their mechanism of intraperitoneal antitumor action before the possible usefulness of these compounds can be assessed.

Based on the intracerebral tumor models employed in this study, these phenothiazines show little promise as single agents for therapy against brain tumors. However, phenothiazines are known to enhance the activity of certain other drugs (7, 9). The very reasonable intraperitoneal antitumor activity of these compounds might suggest a possible use in combination with other agents if the action of the phenothiazines is compatible with a synergistic mechanism.

EXPERIMENTAL[2]

2-Trifluoromethylphenothiazine, 3-chloroethyl-*p*-toluenesulfonate, and 1-piperazineethanol were obtained commercially. When several compounds were prepared by comparable procedures, only one representative example is discussed. Reference should be made to Table I for supplementary information.

Fluphenazine [4-[3-[2-(Trifluoromethyl)phenothiazin-10-yl]-propyl]-1-piperazineethanol] (II)—A stirred mixture of V (52.0 g, 0.15 mole), sodium iodide (22.5 g, 0.15 mole), and 1-piperazineethanol (33.8 g, 0.3 mole) was refluxed in 2-butanone (260 ml) for 20 hr (Scheme I). The mixture was concentrated *in vacuo*, and the residue was poured into a mixture of concentrated hydrochloric acid (40 ml), water (300 ml), and ether (150 ml). After thorough shaking, the aqueous layer was isolated, made basic with 40% aqueous potassium hydroxide, saturated with potassium carbonate, and extracted with three 200-ml portions of ether.

The ether extracts were washed with a saturated sodium chloride solution, dried over sodium sulfate, and concentrated to a dark oil, which was distilled to give 27.2 g (41%) of an oil, bp 260–270°/0.4 torr [lit. (31) bp 268–274°/0.5 torr]. This material was used without further purification for the preparation of VI.

2-[2-[4-[3-[2 - (Trifluoromethyl)phenothiazin-10-yl]propyl]-1-piperazinyl]ethoxy]ethanol Dihydrochloride (III)—Small pieces of sodium (2.3 g, 0.1 mole) were dissolved in ethylene glycol (40 ml) with warming, when necessary, over 3 hr. Compound VI dihydrochloride (9.0 g, 0.017 mole) was added in small portions, and the solution was heated at 105° for 6 hr. After cooling, water (300 ml) was added, and the resulting mixture was extracted with benzene.

The extracts were combined, washed with water, dried over sodium sulfate, and evaporated to a brown oil. The oil was dissolved in dry ether, the solution was filtered, and hydrogen chloride gas was added to yield 8.52 g (90%) of the dihydrochloride salt. Recrystallization from 2-propanol gave a tan solid, mp 222–224° [lit. (23) mp 220–222°].

10-(3-Chloropropyl)-2-(trifluoromethyl)phenothiazine (V)—This compound, mp 73–74° [lit. (32) mp 70–71°], was prepared in a 55% yield on a 35-g scale by the general method of Jackson and Shirley (13).

10-[3-[4-(2-Chloroethyl) -1- piperazinyl]propyl]-2-(trifluoromethyl)phenothiazine Dihydrochloride (VI)—A solution of II (4.58 g, 0.01 mole) in alcohol-free chloroform (100 ml) was saturated with hydrogen chloride gas (Scheme I). To this stirred solution was added dropwise thionyl chloride (2.9 g, 0.02 mole) in purified chloroform (60 ml). A gray precipitate resulted. The stirred reaction mixture was refluxed for 3 hr and then poured into dry ether (300 ml). The resulting fine solid was washed with dry ether and dried to give 5.0 g (95%) of product as a dihydrochloride, mp 208–211° [lit. (23) mp 209–211°].

General Procedure for Phenothiazine Ethers (VII, VIII, and X-XIV)—To a solution of sodium metal (0.23 g, 10 mmoles) in 15 ml of the appropriate alcohol was slowly added 1.22 g (2 mmoles) of VI (Scheme I). The reaction mixture was stirred at reflux for 4 hr. Excess alcohol was removed *in vacuo*, and the residue was treated with water and then extracted with ether. The extracts were washed with saturated aqueous sodium carbonate and then dried over anhydrous sodium carbonate.

Removal of the solvent gave a viscous oil, which was dissolved in benzene and filtered to remove any insoluble material. The benzene was evaporated, the residue was dissolved in dry ether, and hydrogen chloride gas was added. The resulting precipitate was purified by solution in a small amount of methanol and treatment with activated charcoal[3]. After filtration, dry ether was added carefully to reprecipitate the dihydrochloride salt.

General Procedures for Phenothiazine Amines—*Method A (XVI-XVIII)*—A mixture of VI (4.0 g, 7.6 mmoles), sodium iodide (1.0 g, 6.7 mmoles), and 30 ml of the appropriate amine was heated at reflux for 3–6 hr (Scheme I). The excess amine was evaporated *in vacuo*, the residue was treated with aqueous sodium carbonate, and this solution was extracted with benzene. The extracts were dried with sodium sulfate and evaporated to an oily residue, which was dissolved in dry ether. Hydrogen chloride gas was added to precipitate a crude trihydrochloride salt, which was purified by recrystallization from methanol–ether.

Method B (XIX and XX)—A mixture of VI (4.0 g, 7.6 mmoles), sodium iodide (1.0 g, 6.7 mmoles), 50 ml of the appropriate amine, and *N,N*-dimethylformamide (50 ml) was heated at 65–90° for 5 hr (Scheme I). Workup and purification were as described in Method A.

10-[3-[4-[2 - (2-Chloroethoxy)ethyl]-1-piperazinyl]propyl]-2-(trifluoromethyl)-10*H*-phenothiazine Dihydrochloride (IX)—A solution of III (3.2 g, 5.8 mmoles) in purified chloroform (100 ml) was saturated with hydrogen chloride gas. To this stirred, gently heated solution was added dropwise 1.1 g (9.2 mmoles) of thionyl chloride in 25 ml of purified chloroform over 90 min (Scheme II). The mixture was refluxed for an additional 3 hr, treated with activated charcoal, and filtered. The solvent was evaporated and the residue was treated with saturated sodium bicarbonate solution to produce the free base which was extracted with ether. The extracts were dried over anhydrous sodium carbonate.

Addition of hydrogen chloride gas gave 3.4 g of a crude hydrochloride salt. This material was purified by conversion to the free base, which was chromatographed on silica gel with benzene–acetone (1:1) to give 2.2 g (66%) of IX after conversion to the dihydrochloride salt. Recrystallization from methanol–ether gave fine crystals, mp 170.5–173°. The spiro compound XXIII was isolated from a second chromatographic fraction (see XXIII).

10-[3-[4-[2-(2,2-Dimethylhydrazino)ethyl -1- piperazinyl]propyl]-2-(trifluoromethyl)]-10*H*-phenothiazine Trihydrochloride Dihydrate (XXI)—A solution of VI (1.5 g, 2.8 mmoles) in benzene (100 ml) was added to a suspension of sodium iodide (2.0 g, 12.4 mmoles) in 1,1-dimethylhydrazine (40 g, 670 mmoles), and the resulting mixture was refluxed for 5 hr (Scheme I). Evaporation of the solvent gave an oil, which was treated with water and extracted with benzene and chloroform. Upon removal of the combined solvents *in vacuo*, an oil resulted and was then converted into a trihydrochloride salt with hydrogen chloride gas in methanol. Recrystallization from methanol–ether gave 0.6 g (38%) of product, mp 202–205°.

1-[2-[4-[3-[2-(Trifluoromethyl) -10*H*-phenothiazin-10-yl]propyl]-1-piperazinyl]ethyl]pyridinium *p*-Toluenesulfonate (XXII)—*p*-Toluenesulfonyl chloride (1.0 g, 5.25 mmoles) was added portionwise to a cold solution of II (1.6 g, 3.6 mmoles) in dry pyridine (30 ml). After 18 hr at 4°, the mixture was poured into ice water and extracted with ether. The ether extracts were washed first with a 5% sodium bicarbonate solution and then several times with cold water. The ether solution was dried (sodium sulfate) and evaporated to yield an oil; this oil solidified to give 1.4 g of crude product after trituration with ether and benzene. Three recrystallizations from benzene gave colorless crystals, mp 135–137°.

9-[3-[2-(Trifluoromethyl) -10*H*-phenothiazin- 10-yl]propyl]-3-oxa-9-aza-6-azoniaspiro[5.5]undecane Chloride (XXIII)—Continued chromatographic elution of the sample described for IX gave a second fraction containing 0.45 g (13%) of XXIII, mp 238–240°.

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GLC Determination of the Polyvalent Saluretic Uricosuric Agent (2-Cyclopentyl-6,7-dichloro-2-methyl-1-oxo-5-indanyloxy)acetic Acid in Biological Fluids

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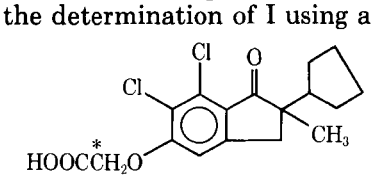
Abstract □ A specific and quantitative GLC method was developed for the determination of (2-cyclopentyl-6,7-dichloro-2-methyl-1-oxo-5-indanyloxy)acetic acid, a novel saluretic uricosuric agent, in biological fluids. The procedure involves the addition of an internal standard to the biological specimens followed by extraction of the acids into benzene at pH 1. The extracted acids, following back-extraction into base and reextraction into methylene chloride at an acidic pH, are converted to the respective methyl esters by reaction with ethereal diazomethane. The sensitivity of the method is such that 2 μg of material can be detected per aliquot of plasma or urine. In the 2.5–50-μg/ml range, recoveries were 98.1 ± 9.6% (plasma, n = 157) and 99.3 ± 6.4% (urine, n = 181). GLC–mass spectrometric techniques established analysis specificity.

Keyphrases □ (2-Cyclopentyl -6,7- dichloro-2-methyl-1-oxo-5-indanyloxy)acetic acid—GLC analysis in biological fluids □ GLC—analysis, (2-cyclopentyl-6,7-dichloro-2-methyl-1-oxo-5-indanyloxy)acetic acid in biological fluids □ Uricosuric agents—(2-cyclopentyl-6,7-dichloro-2-methyl-1-oxo-5-indanyloxy)acetic acid, GLC analysis in biological fluids □ Indanyloxyacetic acid, substituted—GLC analysis in biological fluids

(2-Cyclopentyl-6,7-dichloro -2-methyl-1-oxo-5-indanyloxy)acetic acid (I) is a new nonsulfonamide saluretic agent with uricosuric and antihypertensive activities. The chemical syntheses of this agent and other similar analogs were described recently (1–3). To investigate the physiological disposition of this compound in several species, it was essential that an analytical method for I in biological fluids be developed.

This report describes a specific and sensitive GLC method for the determination of I using a related com-



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