jected intraperitoneally for 5 days, starting on the 1st day after tumor transplantation (Fig. 3). On this resistant line, 5-fluorodeoxyuridine had no effect at this level while I still inhibited tumor growth. Obviously, the effects of other nucleoside phosphodiesters whose action also would be aided by high levels of phosphodiesterase in these resistant tumors should be studied.

REFERENCES

(1) K. C. Tsou, S. Aoyagi, and E. E. Miller, *J. Med. Chem.*, **13**, 765(1970).

(2) K. C. Tsou, K. W. Lo, S. L. Ledis, and E. E. Miller, *ibid.*, **15**, 1221(1972).

(3) M. Umeda and C. Heidelberger, *Cancer Res.*, **28**, 2529(1968).

(4) S. S. Cohen, J. G. Flaks, H. D. Barner, M. R. Loeb, and J. Lichtenstein, *Proc. Nat. Acad. Sci. USA*, **44**, 1004(1958).

(5) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265(1951).

(6) K. W. Lo, W. Ferrar, W. Fineman, and K. C. Tsou, *Anal. Biochem.*, **47**, 609(1972).

(7) T. R. Breitman, *Biochim. Biophys. Acta*, **67**, 153(1963).

(8) A. C. Palidini and L. F. Leloir, *Biochem. J.*, **51**, 426(1952).

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GLC Determination of Plasma Concentrations of Phenprocoumon

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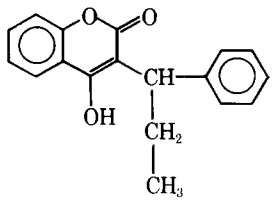
Abstract □ A GLC method for the quantitative estimation of phenprocoumon from plasma is described. Plasma containing phenprocoumon, to which a known amount of phenytoin is added as the internal standard, is acidified and extracted with ethylene dichloride. The drug and the internal standard are then back-extracted into alkali, which is acidified and reextracted with ethylene dichloride. The organic extract is evaporated, and the evaporated residue is mixed with 50 μl of trimethylanilinium hydroxide in methanol. Aliquots (1–2 μl) are injected into a gas chromatograph equipped with a flame-ionization detector in which the injection port is held at 325°. The methyl derivatives of phenprocoumon and the internal standard give sharp, well-separated, symmetrical peaks. The method is of sufficient sensitivity to determine 0.125 μg/ml of the drug in plasma with a coefficient of variation of 7%.

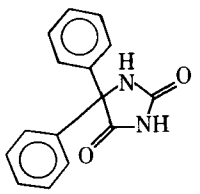
Keyphrases □ Phenprocoumon—GLC determination from human plasma, phenytoin as internal standard □ GLC—analysis, phenprocoumon in plasma, phenytoin as internal standard

Phenprocoumon (I) is an orally administered anticoagulant with action and uses similar to those of phenindione (1). Phenprocoumon is structurally related to warfarin; both are oral coumarin anticoagulants. Several analytical procedures (2–8) were described for the estimation of warfarin and its metabolites from biological fluids, but only one fluorometric method (9) was reported for phenprocoumon. The latter is subject to interference from plasma constituents at low values and from any metabolites present.

The GLC behavior of several coumarin anticoagulants including phenprocoumon, as well as their trimethylsilyl ethers, acetates, trichloroacetates, and trifluoroacetates, was reported (10, 11). These results suggest that derivatization of coumarin drugs might be useful for measuring their concentrations in biological fluids. To facilitate study of the pharmacokinetics and potential interactions with other drugs



I



II

after single- and multiple-dose oral administration of phenprocoumon, a sensitive and specific GLC procedure was developed for the measurement of intact drug in plasma.

EXPERIMENTAL

Reagents—Ethylene dichloride[1] and ether[2] were glass distilled prior to use. Stock solutions containing 100 μg/ml of phenprocoumon[3] were prepared daily in 0.1 *N* NaOH and stored in the dark in a refrigerator. Appropriate dilutions (0.125–4.00 μg/ml) were made immediately before use. Aqueous solutions containing 100 μg/ml of the internal standard, phenytoin[4] (II), were prepared weekly by

[1] Caledon Laboratories Ltd., Georgetown, Ontario, Canada.
[2] Mallinckrodt Chemical Works Ltd., Montréal, Québec, Canada.
[3] Hoffmann-La Roche Ltd., Vaudreuil, Québec, Canada.
[4] USP reference standard.

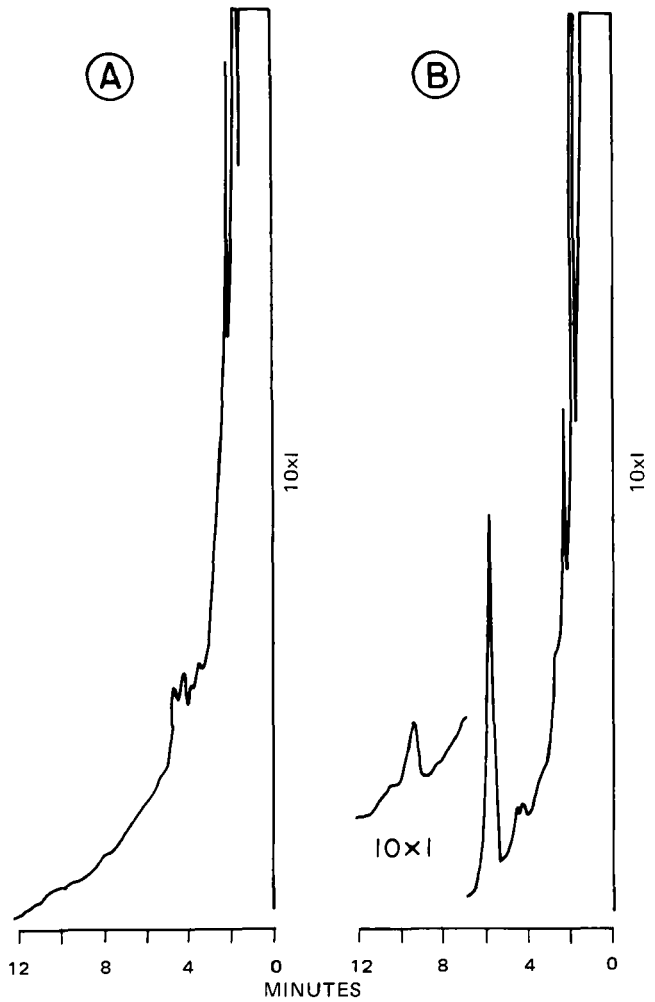
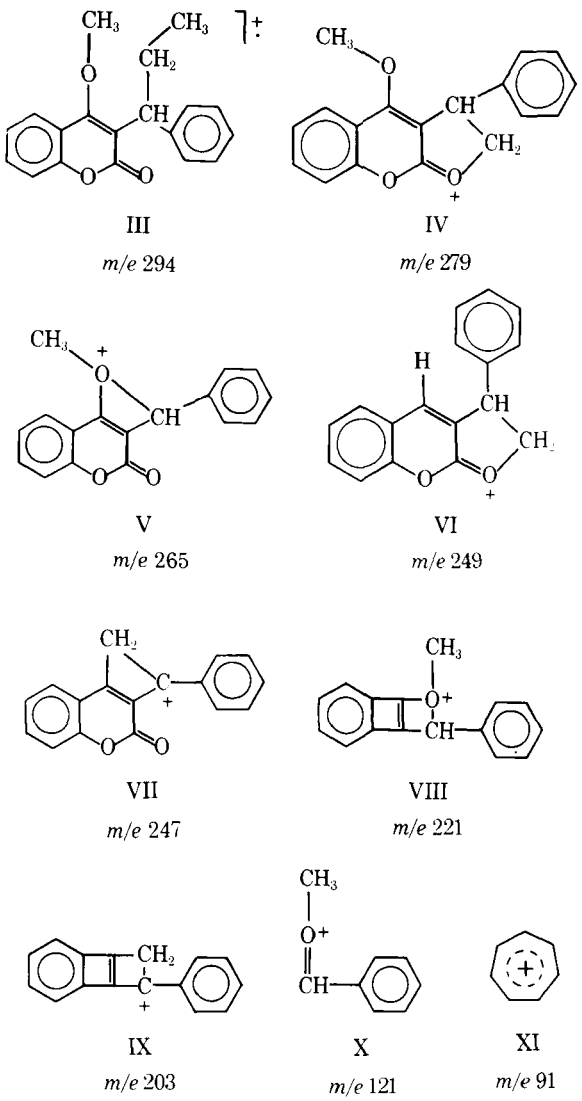


Figure 1—*Gas-liquid chromatograms of human plasma extracts. Key: A, control plasma; and B, plasma spiked with 0.5 μg/ml of phenprocoumon and 2.0 μg/ml of phenytoin.*



dissolving appropriate amounts of the sodium salt of phenytoin and diluted daily to 2 μg/ml with distilled water.

Methanolic trimethylanilinium hydroxide was synthesized according to the method of Barret (12). All other chemicals were analytical grade.

Plasma Level Study—Phenprocoumon (5 mg) was administered separately to two dogs weighing 17.2 and 21.5 kg. Ten-milliliter blood samples were withdrawn from the cephalic vein, using heparinized evacuated tubes[5], at 22 appropriate time intervals over 18 days following dosing. The blood samples were centrifuged, and the plasma was transferred to another tube before storage at −10°.

Extraction of Phenprocoumon—To a 2-ml human or canine plasma sample (spiked or from dosed animals) in a glass-stoppered centrifuge tube[6] (50 ml) were added 1 ml of phenytoin (2.0 μg/ml), 2 ml of 3 *N* HCl, and 20 ml of ethylene dichloride. The samples were stoppered and extracted by shaking at a 30° angle for 10 min at 180 cpm on a flat bed shaker[7]. After centrifugation at 3000 rpm for 10 min, the aqueous layer was removed by aspiration and 18 ml of the ethylene dichloride layer was transferred into another glass-stoppered centrifuge tube[6] (50 ml) containing 10 ml of 1 *N* NaOH.

The extraction was repeated (shaking for 15 min and centrifuging for 10 min), and 9 ml of the sodium hydroxide was transferred to another glass-stoppered tube[6] (50 ml) containing 3 ml of 5 *N* HCl. The tube was swirled gently and the extraction was carried out with another 20 ml of ethylene dichloride for 20 min. The aqueous layer was removed by aspiration, and 18 ml of ethylene dichloride was transferred to a glass-stoppered centrifuge tube[6] (50 ml). The ethylene dichloride was evaporated[8] at 85° under a stream of dry nitrogen to a volume of approximately 4 ml. A crystal of anhydrous calcium chloride[6] was added and the tube was swirled gently. Then the extract was transferred quantitatively into a custom-made evaporation tube (13) containing a small antibumping granule[9] and evaporated as before to dryness.

The tube was allowed to cool to room temperature and the residue was dissolved in 50 μl of methanolic trimethylanilinium hydroxide (0.4 *M*) by mixing[10]. Aliquots (1–2 μl) were injected into the gas chromatograph. The retention times of flash-methylated phenprocoumon and phenytoin were 9.36 and 5.70 min, respectively.

Reference Samples for Recovery—Reference samples were prepared by dissolving appropriate amounts of phenprocoumon and phenytoin in methanol to obtain solutions containing final concentrations of 2 and 4 μg/ml of each drug. One milliliter of methanol solution (2 and 4 μg/ml) of phenprocoumon was pipetted into an evaporating tube containing 1 ml of methanolic solution of phenytoin (2 and 4 μg/ml). The mixture was evaporated to dryness at 85° under a stream of dry nitrogen, and the dried residue was dissolved in 50 μl of methanolic trimethylanilinium hydroxide (0.4 *M*) by means of a vibrating mixer. Aliquots (1–2 μl) were injected into the gas chromatograph, and the peak height ratios thus obtained were designated the 100% value.

[5] Vacutainers, Becton Dickinson and Co., Mississauga, Ontario, Canada.
[6] Fisher Scientific Co. Ltd., Montréal, Québec, Canada.
[7] Eberbach Corp., Ann Arbor, Mich.
[8] Thermolyne Dri-Bath, Fisher Scientific Co. Ltd., Montréal, Québec, Canada.
[9] British Drug House, Toronto, Ontario, Canada.
[10] Vortex Genie, Fisher Scientific Co. Ltd., Montréal, Québec, Canada.

Table I—Recovery of Phenprocoumon and Phenytoin from Plasma[a]

Micrograms Added to 1 ml of Plasma	Mean Micrograms Recovered	Mean Percent Recovery ± *SD*
	Recovery of Phenprocoumon	
2.0	1.72	85.97 ± 1.33
4.0	3.50	87.43 ± 1.10
	Mean = 86.70 ± 1.38%	
	Recovery of Phenytoin	
2.0	1.80	90.18 ± 1.52
4.0	3.61	90.28 ± 0.89
	Mean = 90.23 ± 1.16%	

[a] $n = 4$.

For the recovery experiments of phenprocoumon, drug (2 or 4 μg/ml) was added to plasma and extracted as already described. To the final extract, a methanolic solution of phenytoin (2 μg/ml) was added and the described procedure was followed. The recovery of phenprocoumon was calculated from the peak height ratios thus obtained. Phenprocoumon was used as an external standard in a similar fashion for the recovery experiment with phenytoin.

GLC—A gas chromatograph[11] equipped with a flame-ionization detector was employed. The column was a coil of stainless steel tubing[12], 1.8 m long × 0.3 cm o.d., packed with 5.0% methyl phenyl silicone containing about 75% phenyl silicone[13] coated on acid-washed, dimethylchlorosilane-treated, 80–100-mesh, high performance flux-calcined diatomite support[14].

The column was conditioned by maintaining it at 290° for 18 hr with low nitrogen flow. The injection port, detector, and oven temperatures were 325, 300, and 260°, respectively. Nitrogen, as a carrier gas, was maintained at a flow rate of 60 ml/min. Hydrogen and compressed air flow rates were adjusted to give maximum response.

Calculations—Peak height ratios were calculated by dividing the height of the peak due to methylated phenprocoumon (9.36 min) by that of methylated phenytoin (5.70 min). Calibration curves were constructed from the results of spiked control plasma samples by plotting the peak height ratios against the concentrations of phenprocoumon (micrograms per milliliter of plasma).

RESULTS AND DISCUSSION

Flash-heater methylation of phenprocoumon with trimethylanilinium hydroxide gave a sharp peak with a retention time of 9.36 min (Fig. 1B) under the conditions described under *Experimental*. Flash-heater methylation of phenytoin was reported previously (14, 15). Under the described conditions, the flash-heater methylated derivative of phenytoin gave a sharp symmetrical peak with a retention time of 5.70 min (Fig. 1B); this peak was well resolved from the peak of methylated phenprocoumon.

To establish the structure of the compound giving rise to the peak with a retention time of 9.36 min (Fig. 1) from flash-heater methylation of phenprocoumon, eluates from the GLC column were fed directly into the mass spectrometer[15] and the mass spectrum of the compound giving rise to the peak was recorded. The mass spectrum (Fig. 2) showed a molecular ion of *m/e* 294 and a base peak at *m/e* 265 (V). Other abundant ions were observed at *m/e* 279, 249, 247, 221, 203, 121, and 91. Structures III–XI have been postulated for these ions.

These fragmentations suggest that the flash-heater methylated derivative of phenprocoumon has the structure 3-(α-ethylbenzyl)-4-methoxycoumarin. These observations also confirmed that the intact drug was being measured. The combined GLC–mass spectral evidence obtained for the peak with a retention time of 5.70 min (Fig. 1) derived from flash-heater methylation of the internal standard in the present study was consistent (molecular ion at *m/e* 280 and other diagnostic ions at *m/e* 251, 223, 203, 194, 165, 118, and 77) with the mass spectrum of the dimethylated derivative of phenytoin (14).

Table II—GLC Estimation of Phenprocoumon Added to Plasma

Phenprocoumon Added, μg	*n*	Mean Peak Height Ratio	*SD*	*CV*[a], %
0.125	5	0.033	0.002	6.35
0.250	6	0.058	0.004	7.03
0.5	5	0.122	0.005	3.66
1.0	5	0.238	0.005	1.88
2.0	5	0.468	0.013	2.79
4.0	5	0.946	0.024	2.55

[a] Mean $CV = 4.04\%$; $y = mx$, where $m = 0.236 \pm 0.003$; and $r^2 = 1$.

The use of trimethylanilinium hydroxide as a convenient methylating agent was reported previously (14–17). It offers the advantage that purified plasma extracts do not need to be completely moisture free—a factor that saves time. The reagent is stable over a long period and is easily prepared. The methylation reaction is instantaneous in the injection port at temperatures above 300° (14–17). During the transfer of the final ethylene dichloride extract separated from the acidic solution, it is important to avoid transfer of trace amounts of acid because it was found that in such circumstances methylation with trimethylanilinium hydroxide was not quantitative.

Attempts to prepare trimethylsilyl ethers of phenprocoumon with silylating reagents such as hexamethyldisilazane[16], bis(trimethylsilyl)*trifluoroacetamide*[16], *a mixture of* trimethylsilylimidazole[16], bis(trimethylsilyl)acetamide[16], and trimethylchlorosilane[16], and a mixture of hexamethyldisilazane and trimethylchlorosilane in solvents such as pyridine[16] and acetonitrile[16] were successful, but interference from plasma constituents discouraged this approach.

Various drugs such as phenylbutazone[17], oxyphenbutazone[18], and several barbiturates were investigated as possible internal standards. On flash-heater methylation with trimethylanilinium hydroxide, phenylbutazone gave two peaks as reported earlier (16). The minor peak of methylated phenylbutazone interfered with that of flash-heater methylated phenprocoumon, thereby precluding its use as an internal standard.

Oxyphenbutazone was discarded as a possible internal standard in the assay of phenprocoumon since its flash-heater methylated products (16) had much greater retention times than that of phenprocoumon, thereby increasing the time of analysis. Various barbiturates such as phenobarbital[19], amobarbital[20], and secobarbital[21] could not be employed since the retention times of their flash-heater methylated derivatives were too short. However, since flash-heater methylated phenytoin gave a GLC peak with the desired retention time and had partition characteristics similar to those of phenprocoumon, this compound adequately satisfied the requirements of an internal standard.

Several other liquid phases such as saturated hydrocarbon lubricant[22], *methyl silicone*[23], *phenyl methyl dimethyl silicone*[24], and phenyl methyl (50:50) silicone[25] were tested, but a peak from endogenous material in the plasma interfered with the peak of flash-heater methylated phenprocoumon.

Refrigerated plasma samples containing phenprocoumon were observed to be stable for several weeks. However, evaporated extracts to which trimethylanilinium hydroxide had been added were unstable over 12 hr. The tubes containing evaporated extracts, when stored overnight at −15°, showed lesser amounts (4–5%) of

[11] Model F/11, Perkin-Elmer, Montréal, Québec, Canada.
[12] Chromatographic Specialties, Brockville, Ontario, Canada.
[13] OV-25, Chromatographic Specialties, Brockville, Ontario, Canada.
[14] Chromasorb W, Chromatographic Specialties, Brockville, Ontario, Canada.
[15] Perkin-Elmer model 900 gas chromatograph attached to a Hitachi Perkin-Elmer model RMSU mass spectrometer through a jet-separator.
[16] Pierce Chemical Co., Rockford, Ill.
[17] Butazolidin, Geigy Pharmaceuticals, Montréal, Québec, Canada.
[18] Ciba-Geigy, Dorval, Québec, Canada.
[19] Jules R. Gilbert Ltd., Toronto, Ontario, Canada.
[20] Eli Lilly and Co. Ltd., Toronto, Ontario, Canada.
[21] May & Baker Co. Ltd., Dagenham, Essex, England.
[22] Apiezon-L, Apiezon Products Ltd., London, England.
[23] OV-1, Chromatographic Specialties, Brockville, Ontario, Canada
[24] OV-7, Chromatographic Specialties, Brockville, Ontario, Canada.
[25] OV-17, Chromatographic Specialties, Brockville, Ontario, Canada.

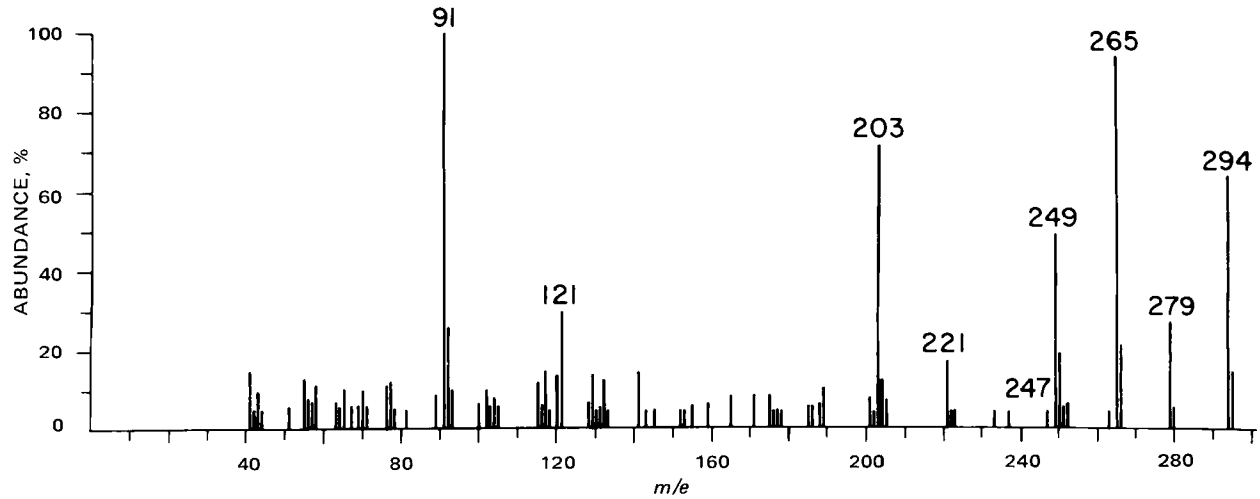


Figure 2—*GLC–mass spectrum (normalized) of flash-methylated phenprocoumon.*

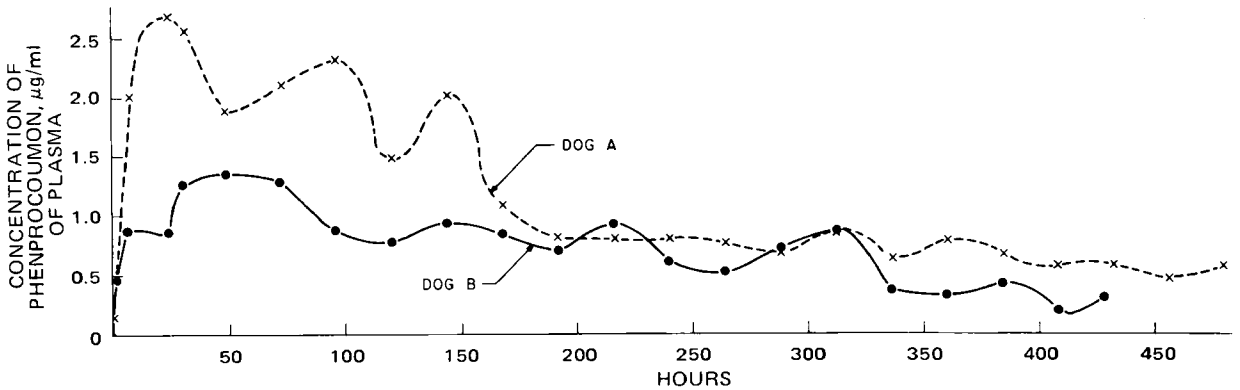


Figure 3—*Plasma profiles of phenprocoumon. Dog A (17.2 kg) and Dog B (21.5 kg) each received 5 mg of phenprocoumon orally in a capsule.*

phenprocoumon. Thus, extraction and GLC analysis of phenprocoumon should be completed on the same day.

Figure 1A shows a typical chromatogram obtained by processing fresh blank plasma as described under *Experimental.* The extraneous peaks near the solvent front were observed in chromatograms of all human plasma samples but not in those of dog plasma samples. A chromatogram obtained when the method was applied to spiked plasma containing 0.5 μg/ml of phenprocoumon and 2.0 μg/ml of phenytoin is shown in Fig. 1B. The extraneous peaks close to the solvent front clearly do not interfere with the peak from the internal standard (retention time of 5.70 min) or phenprocoumon (retention time of 9.36 min).

The response of the flame-ionization detector to flash-heater methylated phenprocoumon was linear with concentration in the 0.125–4.00-μg/ml range. The peak height ratio of the drug and internal standard was used as the index of detector performance and overall efficiency of the analytical procedure. The overall recoveries of phenprocoumon and phenytoin from plasma were in the order of 86.70 ± 1.38 and 90.23 ± 1.16%, respectively (Table I).

The accuracy and precision of the GLC assay are demonstrated in Table II. Results are based on at least five determinations of each phenprocoumon concentration, ranging from 0.125 to 4.00 μg/ml, which were treated as described under *Experimental.* The overall coefficient of variation was 4.04%. The calibration curve obtained by plotting the peak height ratio of flash-heater methylated phenprocoumon/phenytoin *versus* the concentration of phenprocoumon was linear ($y = mx$) over the concentration range of 0.125–4.00 μg of phenprocoumon/ml of plasma. A mean slope value of 0.236 ± 0.002 ($p = 0.05$, $r = 0.9997$) was obtained. When the equation $y = mx + b$ was used for calculations, the slope value was 0.2356 ± 0.0025 ($r = 0.9994$) while the intercept ($b = 0.001457$) obtained was not significant ($p = 0.05$).

Application of the method to plasma level determinations in dogs is demonstrated in Fig. 3. Dogs A and B, 17.2 and 21.5 kg, respectively, each received 5 mg of phenprocoumon orally in an extemporaneously prepared capsule[26]. The many peaks and valleys in the plasma profile remain unexplained and investigations continue. The data provided by Seiler and Duckert (9, 18) for humans do not allow full evaluation, although some scatter is evident from the profiles given.

The described GLC procedure is sensitive for the determination of phenprocoumon, and the plasma samples required are not large. The procedure also offers the advantage that the methyl derivative of the intact drug is measured. It should be suitable for single- or multiple-dose pharmacokinetic studies.

REFERENCES

(1) "Martindale—The Extra Pharmacopoeia," 26th ed., The Pharmaceutical Press, London, England, 1974, p. 874.

(2) R. A. O'Reilly, P. M. Aggeler, M. S. Hoag, and L. Leong, *Thromb. Diath. Haemorrh.*, **8**, 82(1962).

(3) R. A. O'Reilly, P. M. Aggeler, and L. S. Leong, *J. Clin. Invest.*, **42**, 1542(1963).

(4) R. J. Lewis and L. P. Ilnicki, *Clin. Res.*, **17**, 332(1969).

(5) P. G. Welling, K. P. Lee, U. Khanna, and J. G. Wagner, *J. Pharm. Sci.*, **59**, 1621(1970).

(6) E. S. Vessel and C. A. Shively, *Science*, **184**, 466(1974).

(7) D. G. Kaiser and R. S. Martin, *J. Pharm. Sci.*, **63**, 1579(1974).

(8) K. K. Midha, I. J. McGilveray, and J. K. Cooper, *ibid.*, **63**, 1725(1974).

(9) K. Seiler and F. Duckert, *Thromb. Diath. Haemorrh.*, **19**, 389(1968).

[26] Gelatin capsules No. 2, Eli Lilly & Co., Indianapolis, Ind. No excipients were added.

(10) F. W. Deckert, *J. Chromatogr.*, **64**, 201(1972).
(11) *Ibid.*, **64**, 355(1972).
(12) M. J. Barret, *Clin. Chem. Newslett.*, **3**, 16(1971).
(13) A. H. Beckett, *Dan. Tidssk. Farm.*, **40**, 197(1966).
(14) R. H. Hammer, B. J. Wilder, R. R. Streiff, and A. Mayersdorf, *J. Pharm. Sci.*, **60**, 327(1971).
(15) J. MacGee, *Anal. Chem.*, **42**, 421(1970).
(16) K. K. Midha, I. J. McGilveray, and C. Charette, *J. Pharm. Sci.*, **63**, 1234(1974).
(17) *Ibid.*, **63**, 1244(1974).
(18) K. Seiler and F. Duckert, *Thromb. Diath. Haemorrh.*, **21**, 320(1969).

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Anticonvulsant Activity and Selective Inhibition of Nicotinamide Adenine Dinucleotide-Dependent Oxidations by 10-(2-Arylimino-3-acetylamino-4-thiazolidonyl)phenothiazines

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Abstract □ Several 10-(1-acetyl-4-arylthiosemicarbazido)phenothiazines and their corresponding cyclized 10-(2-arylimino-3-acetylamino-4-thiazolidonyl)phenothiazines were synthesized and characterized by their sharp melting points and elemental analyses. All compounds inhibited nicotinamide adenine dinucleotide (NAD)-dependent oxidation of pyruvate and α-ketoglutarate selectively, whereas NAD-independent oxidation of succinate remained unaltered. All phenothiazine derivatives exhibited anticonvulsant activity, which was reflected by the 20–60% protection observed against pentylenetetrazol-induced convulsions in mice. The ability of substituted thiosemicarbazidophenothiazines to inhibit cellular respiratory activity was reduced considerably by cyclization to the corresponding substituted thiazolidinophenothiazines. On the other hand, cyclization generally resulted in increased anticonvulsant activity. Thus, the anticonvulsant activity possessed by these substituted phenothiazines bore no relationship with their ability to inhibit selectively the NAD-dependent oxidations. Selective inhibition of NAD-dependent oxidation of pyruvate and α-ketoglutarate in isolated rat brain mitochondria by some 10-(1-acetyl-4-arylthiosemicarbazido)phenothiazines was concentration dependent and competitive in nature.

Keyphrases □ Phenothiazines—synthesis, effects on NAD-dependent oxidations, anticonvulsant activity screened, mice □ Oxidation, NAD dependent—pyruvate and α-ketoglutarate, effect of phenothiazines □ Anticonvulsant activity—phenothiazines, synthesized, screened, mice □ Structure–activity relationships—substituted phenothiazines, anticonvulsant activity

The striking clinical success of chlorpromazine as an antipsychotic agent led to the synthesis of numerous phenothiazine derivatives. Chlorpromazine has been shown to diminish convulsant effects of nicotine and cocaine, whereas no protection was provided by chlorpromazine against convulsions induced by the administration of strychnine, caffeine, and pentylenetetrazol (1, 2). Earlier studies indicated that small differences in the chemical structure of the phenothiazine compounds produce significant changes in their pharmacological properties (3).

Furthermore, the ability of thiazolidone derivatives to afford protection against pentylenetetrazol-induced convulsions (4, 5) and of phenothiazines and thiazolidones to inhibit cellular respiratory activity of brain homogenates (4, 6) prompted synthesis of phenothiazine derivatives possessing the thiazolidone moiety at position 10 of the phenothiazine portion of their molecular structure. Anticonvulsant activity of these substituted thiazolidonylphenothiazines and their precursor thiosemicarbazidophenothiazines was determined against pentylenetetrazol-induced convulsions in an attempt to show the effect of cyclization on the anticonvulsant activity of these phenothiazines.

The ability of these substituted phenothiazines to inhibit cellular respiratory activity of brain homogenates was also investigated with a view to study the biochemical mechanism of action of these compounds. The various 10-(2-arylimino-3-acetylamino-4-thiazolidonyl)phenothiazines were synthesized as outlined in Scheme I.

EXPERIMENTAL[1]

10-Chloroacetylphenothiazine—Following the method of Ekstrand (7), a mixture of phenothiazine (0.3 mole) and chloroacetyl chloride (0.45 mole) in 100 ml of dry benzene was refluxed on a steam bath for 4 hr. Excess benzene was removed by distillation.

[1] All compounds were analyzed for their carbon, hydrogen, and nitrogen content. Melting points were taken in open capillary tubes and are corrected. IR spectra were obtained with Perkin-Elmer Infracord spectrophotometer model 137 equipped with sodium chloride optics in potassium bromide films in the 700–3500-cm^{-1} range.