on flocculation behavior depends not only on the surfactant concentration but also on the location of the surfactant and the degree of surface coverage by the surfactant. Electrolyte effects at one surfactant level can be eliminated or even reversed by changing the surfactant concentration.

It is also suggested that aggregation in a secondary potential energy minimum as described by the DLVO theory is a valid mechanism for interpreting flocculation. Flocculation mechanisms would then be classified as: (*a*) adsorption bridging, (*b*) cross-linking or chemical bridging, and (*c*) DLVO aggregation (secondary minimum). And coagulation would be defined by: (*a*) film–film bonding, and (*b*) DLVO aggregation (primary minimum).

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Synthesis and Antifungal Activity of Polyhalophenyl Esters of Nitrocarbanilic Acids VI

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Abstract □ Twenty-two polyhalophenyl esters of *meta*- and *para*-nitrocarbanilic acids were synthesized and tested for antifungal activity against *Candida albicans*, *Penicillium notatum*, and *Aspergillus fumigatus*. The 2,4,6-trichlorophenyl ester of *m*-nitrocarbanilic acid was found to be the most active compound.

Keyphrases □ Nitrocarbanilic acids, polyhalophenyl esters—synthesis, antifungal activity □ Polyhalophenyl esters, nitrocarbanilic acids—synthesis, antifungal activity □ Antifungal agents, potential—synthesis and testing of 22 polyhalophenyl esters of nitrocarbanilic acids

In continuing research with the syntheses of new carbamic acid derivatives (1–5) with antifungal activity, a series of polyhalophenyl esters of *meta*- and *para*-nitrocarbanilic acids was prepared by interaction (Scheme I) of the corresponding nitrobenzoyl azide

$$NO_2C_6H_4CON_3 + ArOH \xrightarrow{\text{refluxing toluene}} NO_2C_6H_4NHCO_2Ar + N_2$$

Scheme I

with polyhalophenols in refluxing toluene, and physical data were compiled (Table I).

All prepared compounds were tested against *Candida albicans* 28012, *Penicillium notatum* S-13, and *Aspergillus fumigatus* CDC 24[1] *in vitro* using BBL Sabouraud dextrose agar medium.

Each compound was tested at 5, 10, and 25 mcg./ml. All compounds were dissolved in acetone at a concentration of 5 mg./ml. These solutions were diluted with hot culture medium to the desired concentrations and autoclaved at 120° for 2 hr. Five replicates of each concentration were prepared.

The antifungal activity of all compounds tested except XIX and XX was insignificant at a concentration of 5 mcg./ml. Compounds XII and XV showed only slight growth inhibition at concentrations of 25 mcg./ml. (Table II).

EXPERIMENTAL[2]

m-Nitrocarbanilic acid *p*-tolyl ester was prepared as follows. *m*-Nitrobenzoyl azide (1.92 g., 0.01 mole) in 20 ml. of dry toluene was refluxed for 2 hr. To the boiling mixture, *p*-cresol (1.08 g., 0.01

[1] Microorganisms were obtained from the Department of Parasitology, Public Health Institute, Tehran, Iran.

[2] Melting points were taken on a Kofler hot stage microscope and are uncorrected. The IR spectra were determined with a Leitz spectrograph model III. NMR spectra were obtained on a Varian A 60A instrument. Mass spectra were recorded on a Varian Mat 111 instrument. *m*- and *p*-Nitrobenzoyl azides were prepared according to Curtius *et al.* (6).

Table I—Physical Constants for Polyhalophenyl Esters of Nitrocarbanilic Acids $O_2NC_6H_4NHCO_2R$

Compound Number	Position of Nitro group	R	Yield, %	Melting Point	Formula	Analysis, % Calc.	Found
I	*meta*	*p*-Tolyl	70	135°	$C_{14}H_{12}N_2O_4$	C 61.76	61.69
						H 4.41	4.32
II	*meta*	*p*-Nitrophenyl	55	192°	$C_{13}H_9N_3O_6$	C 51.48	51.42
						H 2.96	2.96
III	*meta*	*p*-Chlorophenyl	65	126°	$C_{13}H_9ClN_2O_4$	C 53.33	53.15
						H 3.07	3.01
IV	*meta*	*p*-Bromophenyl	52	125°	$C_{13}H_9BrN_2O_4$	C 46.29	46.41
						H 2.67	2.57
V	*meta*	*p*-Iodophenyl	49	130°	$C_{13}H_9IN_2O_4$	C 40.62	40.55
						H 2.34	2.40
VI	*meta*	2,4-Dichlorophenyl	45	142°	$C_{13}H_8Cl_2N_2O_4$	C 47.70	47.78
						H 2.44	2.35
VII	*meta*	2,4,5-Trichlorophenyl	57	184°	$C_{13}H_7Cl_3N_2O_4$	C 43.15	42.98
						H 1.93	1.89
VIII	*meta*	2,4,6-Trichlorophenyl	63	162°	$C_{13}H_7Cl_3N_2O_4$	C 43.15	43.20
						H 1.93	1.91
IX	*meta*	2,4,6-Tribromophenyl	65	189°	$C_{13}H_7Br_3N_2O_4$	C 31.51	31.39
						H 1.41	1.39
X	*meta*	2,4,6-Triiodophenyl	45	211°	$C_{13}H_7I_3N_2O_4$	C 24.52	24.45
						H 1.10	1.12
XI	*meta*	Pentachlorophenyl	55	167°	$C_{13}H_5Cl_5N_2O_4$	C 36.23	36.19
						H 1.16	1.20
XII	*para*	*p*-Tolyl	65	215–216°	$C_{14}H_{12}N_2O_4$	C 61.76	61.80
						H 4.41	4.38
XIII	*para*	*p*-Nitrophenyl	52	132–136°	$C_{13}H_9N_3O_6$	C 51.48	51.39
						H 2.96	2.91
XIV	*para*	*p*-Chlorophenyl	60	175°	$C_{13}H_9ClN_2O_4$	C 53.33	53.29
						H 3.07	3.10
XV	*para*	*p*-Bromophenyl	51	185°	$C_{13}H_9BrN_2O_4$	C 46.29	46.32
						H 2.67	2.53
XVI	*para*	*p*-Iodophenyl	46	198°	$C_{13}H_9IN_2O_4$	C 40.62	40.54
						H 2.34	2.29
XVII	*para*	2,4-Dichlorophenyl	48	186°	$C_{13}H_8Cl_2N_2O_4$	C 47.70	47.74
						H 2.44	2.38
XVIII	*para*	2,4,5-Trichlorophenyl	53	235°	$C_{13}H_7Cl_3N_2O_4$	C 43.15	43.20
						H 1.93	2.01
XIX	*para*	2,4,6-Trichlorophenyl	49	250°	$C_{13}H_7Cl_3N_2O_4$	C 43.15	43.30
						H 1.93	1.85
XX	*para*	2,4,6-Tribromophenyl	61	186°	$C_{13}H_7Br_3N_2O_4$	C 31.51	31.46
						H 1.41	1.45
XXI	*para*	2,4,6-Triiodophenyl	48	195°	$C_{13}H_7I_3N_2O_4$	C 24.52	24.48
						H 1.10	1.15
XXII	*para*	Pentachlorophenyl	53	165°	$C_{13}H_5Cl_5N_2O_4$	C 36.23	36.27
						H 1.16	1.18

Table II—Antifungal Activity[a] of Polyhalophenyl Esters of Nitrocarbanilic Acids

Compound	*P. notatum* 10 mcg./ml.	*P. notatum* 25 mcg./ml.	*C. albicans* 10 mcg./ml.	*C. albicans* 25 mcg./ml.	*A. fumigatus* 10 mcg./ml.	*A. fumigatus* 25 mcg./ml.
I	+	2+	–	–	2+	3+
II	+	2+	–	–	–	+
III	–	2+	–	–	–	2+
IV	–	2+	–	–	–	+
V	–	2+	–	–	–	–
VI	–	4+	–	–	–	2+
VII	5+	5+	5+	5+	5+	5+
VIII	2+	5+	–	5+	2+	5+
IX	+	3+	–	4+	+	3+
X	2+	2+	–	–	2+	2+
XI	+	3+	–	4+	+	3+
XIII	–	+	–	–	–	3+
XIV	–	2+	–	–	–	2+
XVI	–	+	–	–	–	+
XVII	–	3+	–	–	+	2+
XVIII	–	+	–	–	–	+
XIX	3+	5+	–	5+	3+	5+
XX	3+	5+	3+	5+	3+	5+
XXI	2+	4+	2+	5+	3+	4+
XXII	2+	5+	+	5+	+	4+

[a] – = no inhibition, and 5+ = complete inhibition.

mole) was added and heating was continued for 2 hr. After evaporation of the solvent, the residue was crystallized from hot benzene to give 1.63 g. (70%), m.p. 135°, molecular weight 272 (by mass spectroscopy); IR: ν_{max} 3200, 1693, 1530, 1500, 1430, 1360, 1260, 1208, 1024, 893, 840, 814, 742, and 675 cm.$^{-1}$; NMR (CF_3CO_2H): τ8.1 (s, 3H, CH_3), 2–3, 2 (m, 8H, aromatic H), and 1.9 (s, 1H, NH).

All other nitrocarbanilic acid esters were prepared similarly (Table I).

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Rapid, Simultaneous GLC Determination of Phenobarbital, Primidone, and Diphenylhydantoin

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Abstract □ A rapid method is described for the simultaneous determination of phenobarbital, primidone, and diphenylhydantoin. The method gives greater sensitivity and reproducibility for phenobarbital and primidone than the short methods now in use, maintains the efficiency of much longer techniques, and takes only 45 min. from the time the sample is received until the results are reported.

Keyphrases □ Phenobarbital—rapid, simultaneous GLC determination with primidone and diphenylhydantoin □ Primidone—rapid, simultaneous GLC determination with phenobarbital and diphenylhydantoin □ Diphenylhydantoin—rapid, simultaneous GLC determination with phenobarbital and primidone □ GLC—rapid, simultaneous determination of phenobarbital, primidone, and diphenylhydantoin

Since the introduction of the on-column methylating reagents, tetramethylammonium hydroxide (1, 2) and trimethylphenylammonium hydroxide (3), the extraction and GLC analysis of anticonvulsant drugs have been greatly simplified. These reagents are normally used as either a dilute (0.1–0.2 *M*) solution (4, 5) or a concentrated (24%) solution (6, 7) in methanol. The methods published to date concerning two of the three primary anticonvulsants, phenobarbital and primidone, are lacking in sensitivity or reproducibility or the extraction time required is unnecessarily long.

This paper describes a combination of two methods (5, 6) which increases the sensitivity and reproducibility of the analysis of phenobarbital and primidone and also decreases the time necessary to give a clean, efficient extraction.

EXPERIMENTAL

Apparatus—A gas chromatograph[1] with four flame-ionization detectors was used for the determinations. The column was a 1.82-m. (6-ft.) by 2-mm. i.d. glass U-tube packed with 3% OV-17 (phenyl methyl silicone oil) on 80–100-mesh Chromosorb W-HP. The injection port was heated to 360° and the detector to 260°. The column temperature was programmed from 140 to 220° at 8°/min. The carrier gas flow rate was adjusted to around 60 ml./min. to give a retention time of 5.0 ± 0.2 min. for methylated diphenylhydantoin at 220°.

Reagents—Trimethylphenylammonium hydroxide[2] was available as a 0.1 *M* solution in methanol; it was concentrated to 1.5 *M* under dry nitrogen over low heat. 5-(*p*-Methylphenyl)-5-phenylhydantoin[3] (I) and phenylethylmalonamide[4] were also used.

Procedure—One milliliter of plasma, 0.50 ml. of the internal standard (20 mg. I diluted to 500 ml. with 0.1 *N* NaOH), 0.5 ml. 0.1 *N* NaOH, 0.5 ml. of a 1.0 *M* H_3PO_4 buffer, pH 2.7, and 13 ml. of ether were combined in a 50-ml. centrifuge tube. The tube was sealed by wetting the ground-glass portion of the stopper with distilled water before insertion, shaken mechanically for 10 min., and centrifuged at 2000 r.p.m. for 1 min. The ether was transferred to a 15-ml. conical centrifuge tube and evaporated under nitrogen at 50°. The residue was taken up in 5 ml. of toluene, and 50 μl. of 1.5 *M* trimethylphenylammonium hydroxide was added. The tube was shaken for 1 min. and then centrifuged for 2 min.; 1 μl. of the lower phase was injected into the chromatograph.

Drug levels were determined from a standard curve of the peak height ratio of the drug to the internal standard *versus* concentration. Phenobarbital levels were determined by summing the heights of the three peaks shown in Fig. 1 after adjusting to constant peak width. The total phenobarbital (Pb) peak height is given by Eq. 1:

$$h_{Pb} = \frac{w_1}{w_3}h_1 + \frac{w_2}{w_3}h_2 + h_3 \qquad \text{(Eq. 1)}$$

where h is the height and w is the width at half height of the peaks shown by the subscripts. The peak width ratios are constant for any given set of chromatographic conditions.

RESULTS AND DISCUSSION

The chromatogram of an extracted sample containing therapeutic levels of phenobarbital, primidone, and diphenylhydantoin, along with an extraction of standard plasma, is shown in Fig. 1. The first two phenobarbital peaks shown in this figure are produced in the chromatograph during on-column methylation with 1.5 *M* trimethylphenylammonium hydroxide. They are also the only peaks produced by methylation of the total product of the aqueous alkaline hydrolysis of phenobarbital. GLC and NMR studies of these two products indicate that they are methylated derivatives of phenyl-

[1] Varian 2100.

[2] Eastman.

[3] Aldrich Chemical Co.

[4] Supplied by Dr. C. E. Pippenger and Dr. B. B. Gallagher.