Table I—*In Vitro* Dissolution t_{50} *versus* Hardening Time for Undecenovanillylamide Microcapsules [a]

Hardening Time, min	*In Vitro* Dissolution t_{50}, min
Unencapsulated	<3
30	7.3
60	17.7
120	28.0

[a] $r = 0.98149$.

plug to ensure that undissolved drug was not included. An equivalent volume of fresh buffer and the glass wool plug were added to the flask after each withdrawal. The samples were then assayed spectrophotometrically at 280 nm after appropriate dilution with pH 6.5 buffer. Each determination was done at least in triplicate.

RESULTS AND DISCUSSION

Since encapsulation *via* complex coacervation occurs best at low colloid concentrations and when acacia and gelatin are employed in a 1:1 ratio (2), 1–4% concentrations were studied. There were no beneficial aspects at the higher concentration, so the 1% colloid concentrations were used to produce the least cohesive powder. The colloidal silica was added to aid in production and did, in fact, lead to easier microcapsule recovery as a dry, noncohesive powder.

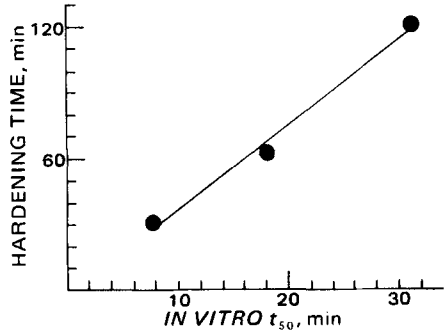

Figure 2—*Hardening time* versus in vitro t_{50} *for undecenovanillylamide microcapsules.*

The resuspension of the product in isopropanol for dehydration was successful with only 30% isopropanol. Lower concentrations did not result in a free-flowing powder, and higher concentrations destroyed the shell wall integrity.

Figure 1 shows the percent release *versus* time data for the various dosage forms studied. Each determination is the average of at least six dissolution profiles. Dissolution results for the powder and encapsulated forms of I indicate that the microencapsulated forms exhibited slower dissolution. The capsules hardened for 0.5 hr featured rapid initial release. This initial release may result from the soft shell and the large amount of unencapsulated I. The profiles in Fig. 1 are in rank order with hardening times; there was a definite delayed, sustained release for encapsulated I.

To explain the drug dissolution from the microcapsules, the release of the active ingredient through leaching or diffusion may be examined. For such release to occur, the dissolution medium must penetrate the wall to the nucleus, dissolve I, and then permeate out through the shell wall. Any of these processes may be rate limiting.

The effect of increased hardening times on the release profile is readily apparent. Since the dissolution t_{50} indicates the central tendency of the data, that point was chosen to compare release (Table I). There was a direct linear correlation ($r = 0.98149$) between microcapsule hardening time and the dissolution t_{50} values (Fig. 2).

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Convenient Synthesis of *N*-Alkoxymethylbarbituric Acids and *N*-Alkoxymethylhydantoins

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Received April 4, 1979, from the *Department of Pharmacology, Division of Clinical Pharmacology, School of Medicine, University of Colorado Medical Center, Denver, CO 80262.* Accepted for publication June 15, 1979.

Abstract □ A simple one-step method for the *N*-alkoxymethylation of barbituric acids and hydantoins is presented. The compounds are *N*-methoxymethylated or *N*-ethoxymethylated using phosphorus pentoxide and dimethoxymethane or diethoxymethane, respectively, in a chlorinated solvent. 1-Methoxymethyl-3-ethyl-5-phenylhydantoin showed significant anticonvulsant activity in the subcutaneous pentylenetetrazol test, while 1,3-bis(methoxymethyl)-5-ethyl-5-(*p*-tolyl)barbituric acid was inactive.

Keyphrases □ *N*-Alkoxymethylbarbituric acids—syntheses, anticonvulsant activity, structure-activity relationships □ *N*-Alkoxymethylhydantoins—syntheses, anticonvulsant activity, structure-activity relationships □ Anticonvulsants—*N*-alkoxymethylbarbituric acids and *N*-alkoxymethylhydantoins, syntheses, structure-activity relationships

N-Alkoxymethyl derivatives of barbituric acids and of hydantoins were recently synthesized, and several derivatives were shown to possess significant anticonvulsant activity in animal tests (1–4). One, 1,3-bis(methoxymethyl)phenobarbital (I), was studied in epileptic patients with promising results (5).

BACKGROUND

The original preparation of the *N*-alkoxymethyl derivatives involved alkylation of the barbituric acid or hydantoin alkali salt with chloromethyl methyl ether (II) (1–4). Since the latter reagent is a potent carcinogen (6–8), its use is highly restricted (9). Therefore, alternatives for the preparation of I have been sought, and two appeared in the patent literature. One method (10) is based on the preparation of 1,3-bis(chloromethyl)phenobarbital from phenobarbital, formaldehyde, acetyl chloride, hydrochloric acid, and a Lewis acid. These conditions are likely (11, 12) to result in the formation of bis(chloromethyl)ether (III), another, even more potent, carcinogen (8). 1,3-Bis(chloromethyl)phenobarbital was converted to I using sodium methoxide in methanol (10). The second

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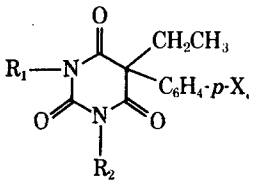
Table I—Products of *N*-Alkoxymethylations

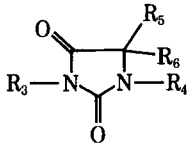
Starting Material	Product	Reaction Time[a]	Yield %	Product: Melting Point[b,c]	Product: NMR[d] Spectrum	Product: Mass Spectrum[e], *m/e* (relative intensity)
V	I	3	83	116–118°[f]	—[g]	—[h]
V[i]	VI	4	56	64–66°[j]	—[g]	—[g]
IX	VIII	3	51	114–115°[k]	—[g]	—[h]
XI	X	3 hr	71	74–76°[l]	7.44–7.22 (m, 5, C_6H_5), 4.64 (*AB* qt, 2, J_{AB} = 12 Hz, ν_A = 4.97, ν_B = 4.32, CH_2O), 3.38 (s, 3, OCH_3), 3.11 (s, 3, NCH_3), 2.89–2.00 (m, 2, CH_2CH_3), 0.96 (t, 3, J = 6 Hz, CH_2CH_3)	262 (1, M)[m], 233 (10), 203 (2), 118 (5), 117 (7), 91 (13), 77 (8), 45 (100), 44 (29)
XIII	XII[n,o]	6 hr	76	55–56°	7.67–7.00 (m, 5, C_6H_5), 4.67 (*AB* qt, 2, J_{AB} = 10 Hz, ν_A = 5.10, ν_B = 4.24, CH_2O), 5.05 (s, 1, CH), 3.64 (qt, 2, J = 6 Hz, CH_3CH_2), 3.33 (s, 3, OCH_3), 1.26 (t, 3, J = 6 Hz, CH_2CH_3)	248 (1, M), 233 (1), 217 (6), 216 (4), 146 (5), 132 (18), 118 (32), 117 (7), 91 (26), 90 (16), 77 (19), 76 (10), 51 (9), 45 (100), 44 (26)
XV	XIV[n,p]	3	87	139–141°	7.19 (s, 4, C_6H_4), 5.38 (*AB* qt, 4, J_{AB} = 10 Hz, ν_A = 5.44, ν_B = 5.29, NCH_2), 3.37 (s, 6, OCH_3), 2.46 (qt, 2, J = 7 Hz, CH_2CH_3), 2.31 (s, 3, $C_6H_4CH_3$), 0.98 (t, 3, J = 7 Hz, CH_2CH_3)	334 (3, M), 302 (10), 274 (11), 202 (9), 201 (6), 190 (15), 188 (17), 174 (5), 161 (15), 160 (100), 159 (14), 146 (6), 132 (26), 131 (26), 117 (57), 115 (17), 105 (12), 91 (18), 86 (29)
V	XVI	3	9	88–89°[q]	—[g]	—[g]

[a] In days, unless otherwise indicated. [b] Determined in a Fisher–Johns apparatus; values are uncorrected. [c] Product recrystallized from ethanol–water. [d] Determined with a Varian Associates EM-360 or EM-390 instrument, recorded in deuterochloroform; data are reported in parts per million (σ) downfield from tetramethylsilane internal standard. [e] Determined on Finnigan 1015 mass spectrometer, ionizing energy 70 ev. [f] Lit. (1) mp 116–118°. [g] Essentially identical to spectrum of same compound prepared *via* original procedure (1–4). [h] Essentially identical to published mass spectrum (16). [i] Diethoxymethane (VII) used in place of IV. [j] Lit. (1) mp 65–66°. [k] Lit. (3) mp 115–116°. [l] Lit. (4) mp 68.5–70.5°. [m] M = molecular ion. [n] Elemental analysis performed by Galbraith Laboratories, Knoxville, Tenn. [o] Calculated for $C_{13}H_{16}N_2O_3$: C, 62.89; H, 6.50; N, 11.28. Found: C, 62.95; H, 6.55; N, 11.25. [p] Calculated for $C_{17}H_{22}N_2O_5$: C, 61.07; H, 6.63; N, 8.38. Found: C, 60.89; H, 6.82; N, 8.25. [q] Melting point of sample prepared *via* previously published method (2): 88–89°.

alternative for the preparation of I involved the reaction of stannic chloride and dimethoxymethane (IV) with phenobarbital (V) (13). The presence of stannic chloride, a Lewis acid, in IV, however, carries the danger of generating chloromethyl methyl ether. Supporting this possibility is the facile conversion of IV to chloromethyl methyl ether in the presence of dry hydrogen chloride (14) or acetyl chloride (15).



	R_1	R_2	X
I	CH_3OCH_2	CH_3OCH_2	H
V	H	H	H
VI	$CH_3CH_2OCH_2$	$CH_3CH_2OCH_2$	H
VIII	CH_3OCH_2	CH_3	H
IX	H	CH_3	H
XIV	CH_3OCH_2	CH_3OCH_2	CH_3
XV	H	H	CH_3
XVI	CH_3OCH_2	H	H



	R_3	R_4	R_5	R_6
X	CH_3	CH_3OCH_2	CH_2CH_3	C_6H_5
XI	CH_3	H	CH_2CH_3	C_6H_5
XII	CH_3CH_2	CH_3OCH_2	H	C_6H_5
XIII	CH_3CH_2	H	H	C_6H_5

YCH_2OCH_2Z

	Y	Z
II	H	Cl
III	Cl	Cl
IV	H	OCH_3
VII	CH_3	OCH_2CH_3

It appears, therefore, that an alternative method not involving carcinogenic halomethyl ethers is needed for the synthesis of *N*-alkoxymethyl derivatives of barbituric acids and hydantoins. Such a method is reported here.

EXPERIMENTAL

1,3-Bis(methoxymethyl)phenobarbital (I)—Phenobarbital (V, 500 mg, 2.2 mmoles), phosphorus pentoxide powder[1] (8 g), and methylene chloride (8 ml) were placed in a 125-ml, single-necked round-bottom flask. Compound IV (8 ml) was added, the flask was capped with a calcium chloride drying tube, and the mixture was stirred for 3 days. The flask was cooled in an ice bath while water (10 ml) was added. The flask was allowed to warm to room temperature, with occasional cooling in ice to control the reaction between water and phosphorus pentoxide.

Ether (10 ml) was added, and the mixture was transferred to a separator. The layers were separated after extraction, and the aqueous layer was further extracted with ether (2 × 10 ml). The combined organic extracts were washed with 1 *N* NaOH (2 × 10 ml) and with water (2 × 10 ml). The solution was dried (sodium sulfate), and the solvent was evaporated to obtain 579 mg of I. Further experimental details are listed in Table I.

Compounds VI, VIII, X, XII, and XIV also were prepared using this procedure, with some variation in experimental details (Table I).

1-Methoxymethylphenobarbital (XVI)—Phenobarbital (V, 2.0 g, 8.6 mmoles), methylene chloride (30 ml), and IV (30 ml) were combined in a 125-ml flask. The mixture was stirred magnetically while phosphorus pentoxide (20 g) was added in small portions. The flask was capped with a calcium chloride drying tube, and the mixture was stirred at room temperature for 3 days. The reaction flask was cooled in ice, and ice water (50 ml) was added with stirring until all of the phosphorus pentoxide had dissolved.

The mixture was extracted with ether (2 × 50 ml). The combined organic layers were extracted with 0.1 *N* NaOH (3 × 50 ml), and the combined alkaline aqueous layers were acidified to pH 4 with concentrated hydrochloric acid in an ice bath. The acidic solution was extracted with ether (3 × 50 ml). The combined organic extracts were washed with water (50 ml), dried with sodium sulfate, and concentrated *in vacuo*. The residue was kept at 4° for 3 days. The resulting crystalline material was recrystallized from ethanol–water (Table I).

Pharmacological Testing—Anticonvulsant activity was examined[2]

[1] Granular phosphorus pentoxide was much less effective.

[2] The testing was carried out by the Antiepileptic Drug Development Program, Epilepsy Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD 20014.

Table II—Anticonvulsant Activity and Neurotoxicity [a]

Compound	MES[b]		scMet[b]		Toxicity[b]	
	0.5 hr	4 hr	0.5 hr	4 hr	0.5 hr	4 hr
XII	+	–	++[c]	–	+	–
XIV	–	–	–	+[d]	–	–

[a] Assay methods are described under *Experimental*. [b] Activity and toxicity at 30, 100, or 300 mg/kg are represented by +++, ++, or +, respectively; – indicates no activity or toxicity observed. [c] Test repeated at 100 mg/kg: three of four animals protected. [d] Test repeated at 300 mg/kg: no animals protected.

in Screen 1 of the Anticonvulsant Screening Project Test Systems (17, 18). Three tests were performed in male mice[3]: the maximal electroshock seizure test (MES), the subcutaneous pentylenetetrazol seizure threshold test (scMet), and the rotorod test designed to detect neurotoxicity.

The compounds were tested at three dose levels (30, 100, and 300 mg/kg) at 30 min and 4 hr after their intraperitoneal administration. Four animals were injected with each dose, which was solubilized in 30% polyethylene glycol 400, and were examined 30 min later for toxicity in the rotorod test. Anticonvulsant activity was evaluated immediately thereafter by subjecting one mouse to the maximal electroshock seizure test and another to the subcutaneous pentylenetetrazol seizure threshold test. The same tests were repeated after 4 hr on the two remaining mice.

Anticonvulsant activity in the maximal electroshock seizure test is defined as abolition of the hindlimb tonic extensor component of the maximal electroshock seizure elicited with a 60-Hz alternating current of 50 mamp delivered for 0.2 sec *via* corneal electrodes. Activity in the subcutaneous pentylenetetrazol seizure threshold test is defined as failure to observe even a threshold seizure (a single episode of clonic spasms at least 5 sec in duration) at a pentylenetetrazol dose of 85 mg/kg. Neurologic toxicity is defined as failure of an animal to remain for at least 1 min on a 2.54-cm diameter, knurled plastic rod rotating at 6 rpm.

RESULTS AND DISCUSSION

The methoxymethylation of alcohols using IV and phosphorus pentoxide was described recently (19). With a related procedure, other investigators methoxymethylated phenols using IV and *p*-toluenesulfonic acid in the presence of molecular sieves (20). The applicability of these methods to the alkoxymethylation of barbituric acids and hydantoins was examined. While the latter procedure (20) failed to methoxymethylate any of the compounds studied, the former method (19) resulted in moderate to excellent yields of alkoxymethylated products (Table I).

Compounds I, VI, VIII, X, XII, XIV, and XVI were prepared. The melting point of X, 74–76°, differed slightly from the previously reported (4) value, 68.5–70.5°. This discrepancy may be because X was not recrystallized in the previous procedure (4). The NMR and mass spectra of X (Table I) were consistent with the proposed structure. Spectral data, melting points, and analytical data (for new compounds) also confirmed the structures of the other compounds. The methylene protons of the *N*-alkoxymethyl group in all of the compounds prepared gave rise to *AB* quartets in the NMR spectra. The reaction proceeded best in a halogenated solvent, *e.g.*, methylene chloride, chloroform, or ethylene dichloride. However, the reaction also was carried out successfully in other solvents. For example, I was prepared in ethyl acetate in a 77% yield.

The procedure also was useful in the preparation of 1-methoxymethylphenobarbital (XVI). Since XVI, an *N*-monosubstituted barbituric acid, could not be prepared by monomethoxymethylation of V with chloromethyl methyl ether (1), an alternative synthesis was developed (2) involving the *N,S*-bis(methoxymethyl) derivative of thiophenobarbital. The method uses chloromethyl methyl ether and is time consuming (2). The reaction described in the present report can be used to prepare XVI in moderate amounts in one safe, simple step.

Compounds XII and XIV, previously unreported, were tested for anticonvulsant activity (Table II). Compound XIV showed no anticonvulsant activity in the test system. This result is interesting when contrasted to the high activity (1) shown by I, a compound differing from XIV only in the lack of the *p*-methyl group on the phenyl ring. Compound XII, the 1-methoxymethyl derivative of ethotoin, showed activity at 100 mg/kg in the subcutaneous pentylenetetrazol test and at 300 mg/kg in the maximal electroshock seizure test at 30 min after administration. However, at 300 mg/kg, XII also had neurotoxic effects.

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