	II	III
Appearance	Greenish-yellow crystals	Greenish-yellow crystals
Melting point (uncorrected)	81–84°	105–107°
λ_{max} in ethanol	345 nm. (ϵ 4100) 283 s (4590) 253 (15,860)	345 nm. (ϵ 3780) 283 s (3880) 253 (14,800) 215 s (46,000)
λ_{max} excitation	350 nm.	350 nm.
λ_{max} emission	530 nm.	530 nm.

is substituted into the nucleophilic 2-position of the aromatic ring (4, 5). On NMR analysis in deuteriochloroform, the dansylation products of Δ⁹- or Δ⁸-tetrahydrocannabinol exhibit two doublets, each integrating for one proton, in the aromatic region (II, δ = 6.45 p.p.m., 6.22; III, δ = 6.50 p.p.m., 6.27) with a coupling constant of 1.5–2.0 Hz. These signals fit an *AB* system characteristic of *meta*-coupled aromatic protons. The more downfield proton corresponds to the 4-position proton and the other to the 2-position proton. On this basis, II and III are proposed to be *O*-dansyl and not C-dansyl compounds. The absence of any exchangeable protons upon the addition of D_2O provides further evidence to support the proposed structure.

Dansylated compounds were reported (1) to decompose when allowed to remain on silica gel for prolonged periods. In our experience, if pure II was left on a silica gel thin-layer plate for over 4 hr. in a dry state, the original greenish-yellow fluorescence of the spot took on a dull-orange color. Scraping and rechromatographing this spot gave at least two additional spots. This observation suggests that II undergoes significant degradation under the test conditions. The literature further documents examples of instability of dansylated phenols to UV exposure (6). Irradiation of Compounds II and III on a silica gel plate with 350- or 254-nm. UV light caused changes in TLC properties after only 10 min.

Solutions of II and III in ethanol slowly decompose regardless of storage conditions. After storage at room temperature and exposure to laboratory light, changes in color and in the UV absorption spectrum were apparent after 1 week. Refrigeration delayed these changes for several weeks. However, storage of the crystals in screw-capped vials under nitrogen, in the dark, in a

desiccator produced no observable changes in TLC properties in more than 3 months.

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|	II m/e	Relative Abundance		III m/e	Relative Abundance
M⁺	547	8	M⁺	547	18
M−SO₂	483	3	M−SO₂	483	14
	464	2		464	16
	412	6		412	3
	314	31		313	9
	313	100		231	3
	231	4		171	100
	171	78		170	86
	170	32			

Mechanism of Phenobarbital Degradation

Keyphrases ☐ Phenobarbital—mechanism of degradation, products identified ☐ Barbiturate degradation—mechanism of ring cleavage, phenobarbital and products

Sir:

In a recent paper, Garrett *et al.* (1) further elucidated the kinetics of hydrolysis of several important barbiturates. In their studies, they discovered the rather surprising fact that diethylmalonuric acid (V) (Scheme I) in basic solution may cyclize to form the parent-substituted barbituric acid, barbital (IV). Previous workers (2, 3) assumed that the hydrolysis of the parent barbiturate to the corresponding malonuric acid was irreversible, and various degradation schemes were predicated on that assumption. Hegarty and Bruice (4) also reported a similar reaction in the cyclization of 2-ureidobenzoic acid.

We have now repeated the work relative to diethylmalonuric acid and verified by mass spectrometry that the cyclization product of diethylmalonuric acid in basic solution is barbital. The reversibility of the hydrolysis of the barbituric acid nucleus is an important discovery and may have interesting biological ramifications.
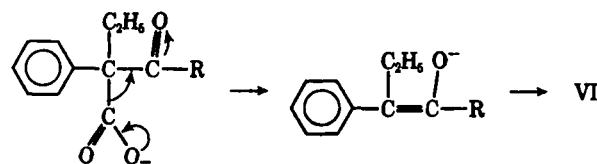
In discussing the reversibility of this reaction, Garrett *et al.* (1) challenged the mechanism of phenobarbital degradation proposed by Tishler *et al.* (3). They put forward a rather tortuous argument to explain the ex-
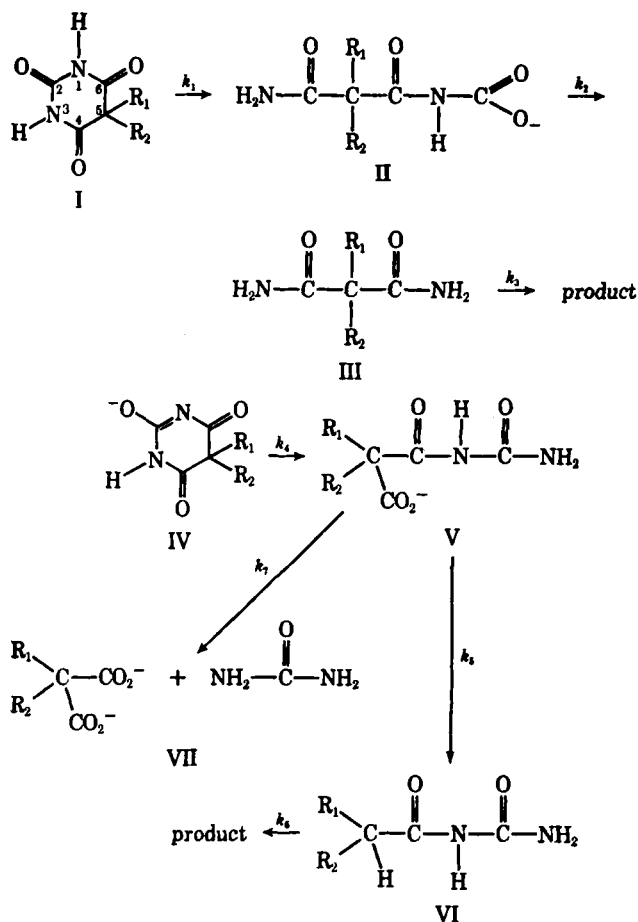
Scheme I—Degradative pathway of barbituric acid derivatives (barbital: R_1, R_2 = ethyl; phenobarbital: R_1 = ethyl, R_2 = phenyl)

Scheme II—Postulated mechanism for decarboxylation of disubstituted malonuric acid

perimental results of Tishler and coworkers. However, their argument depends on the reversibility of the phenylmalonuric acid formation in a manner analogous to diethylmalonuric acid. In the paper where Fretwurst (5) reported recovery of 50% of degraded barbital as the diethylmalonuric acid, he also reported finding no corresponding malonuric acid under several different conditions with phenobarbital. Several other barbiturates, substituted such that the malonuric acid derived from them contains a group that can conjugate with the ureide carbonyl in the enol intermediate postulated for the decarboxylation mechanism (Scheme II), likewise were devoid of isolatable substituted malonuric acid. If the mechanism proposed by Hegarty and Bruice (4) for the cyclization of 2-ureidobenzoic acid is correct, different malonuric acids should cyclize at about the same rate (in basic solution) since the mechanism depends upon the nucleophilicity of the terminal amino group, which would be only minimally affected by different substituents. It would, therefore, appear that substitution of one of the ethyl groups of barbital by the phenyl group results in labilization of malonuric acid, such that decarboxylation occurs at a much higher rate than in the barbital product, with little change in the cyclization rate, making the step from phenobarbital to phenylethylmalonuric acid essentially irreversible, as assumed by Tishler et al. (3).

Moreover, Fretwurst (5), when using two equivalents of sodium hydroxide for each equivalent of phenobarbital, identified 73% of the reaction product as

malonic acid (VII) instead of isolating the substituted acetylurea (VI) as the primary product, apparently indicating that the hydrolysis to malonic acid at higher base concentrations proceeds at a more rapid rate than the parallel decarboxylation reaction.

The known facts relative to barbiturate degradation would fit a scheme in which the unionized barbiturate can be cleaved at the one-two position, leading to production of the bisamide (III), or at the one-six (three-four) position, leading to the ureide (VI); the ionized barbiturate would cleave only at the one-six (three-four) position, leading to the ureide (or malonic acid) exclusively. For example, in the paper by Tishler et al., (3) the equation describing the ureide pathway would be:

$$ f = \frac{k_4 K_a + k_u[H^+]}{k_d[K_a] + k_1[H^+]} \qquad \text{(Eq. 1)} $$

assuming that k_1, mentioned in the previous paper (3), is the sum of the rate constant relating production of the ureide, k_u, and the rate constant relative to the rate of production of the bisamide, k_d, from undissociated phenobarbital (I). In the 8.2–9.5 pH range studied by the authors, the K_a (10^{-7}–10^{-8}) of phenobarbital would be much greater than the hydrogen-ion concentration, and one could assume with Garrett et al. (1) that the ureide pathway for the unionized barbiturate is severely hampered through steric hindrance by the presence of the phenyl group at the five position; thus, the product $k_4 K_a$ would be much greater than $k_u[H^+]$, and the results found by Tishler et al. (3) are explained. On the other hand, the results also reported by Garrett et al. (1) relative to the hydrolysis of barbital at pH 6.15, where the ureide represents 84% of hydrolyzed barbital, is also explicable in allowing the nonsterically hindered hydroxide-ion attack at the one-six or three-four position. It is certainly clear that more work is needed on the hydrolysis of barbiturates, particularly on the degradation beyond the initial cleavage of the barbituric acid itself.

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