Table II—Determinations of Urine Specimens from Humans Administered Sodium Dantrolene by the Nitromethane–Hyamine Method[a]

Subject	Absorbance[b], 400 nm.	Concentration[c], mcg./ml.
3	0.098 (0.062)	3.06
6	0.149 (0.034)	4.65
2	0.719 (0.013)	22.47
5	0.745 (0.015)	23.28
11	0.541 (0.032)	16.90

[a] An encapsulated sodium dantrolene formulation was administered orally as a single dose at 100 mg. to Subjects 3 and 6 and at 100 mg. q.i.d. to Subjects 2, 5, and 11. Urine specimens were collected from 0–24 hr., pooled, and frozen. A control urine specimen was collected just before drug administration. [b] Control corrected; figure in parentheses represents the control urine absorbance. [c] Estimated drug concentration for 0–24 hr., expressed as dantrolene equivalents, represents dantrolene plus Metabolite A.

extracts from each of the specimens, collected after drug administration, exhibited a visual yellow color following the addition of the hyamine reagent (Table II). Under these conditions, a yellow color was not apparent with extracts from corresponding control urines, collected before drug administration.

The dantrolene–hyamine complex in nitromethane exhibits an absorbance maximum near 400 nm., while the corresponding Metabolite A–hyamine complex displays a maximum near 395 nm. When subjected to the nitromethane–hyamine procedure, the human urines collected after the administration of sodium dantrolene yielded an absorbance maximum from 380 to 385 nm.

An estimate of the drug and metabolite concentration present in the urine specimens was obtained by spectrophotometric measurement (Table II). These results are expressed as dantrolene equivalents, based on dantrolene standards, and represent dantrolene plus Metabolite A.

DISCUSSION

At present, two of the major dantrolene-related metabolites recovered from human urine after the administration of sodium dantrolene are reduced acetylated dantrolene and a nonreduced metabolite designated as A (5). The amount of each of these metabolites excreted in urine is usually much greater than the corresponding amount of dantrolene (5). It is assumed that dantrolene plus Metabolite A is primarily responsible for the yellow color observed when urine specimens collected from humans receiving sodium dantrolene are analyzed by the nitromethane–hyamine procedure, since standards of acetylated dantrolene do not exhibit an appreciable absorbance when subjected to the qualitative method. A partial explanation for this specificity is that acetylated dantrolene does not contain the nitro group on the benzene ring which is present in both dantrolene and Metabolite A.

REFERENCES

(1) H. R. Snyder, C. S. Davis, R. K. Bickerton, and R. P. Halliday, *J. Med. Chem.*, **10**, 807(1967).

(2) L. J. Honkomp, R. P. Halliday, and F. L. Wessels, *Pharmacologist*, **12**, 201(1970).

(3) K. O. Ellis and J. F. Carpenter, *Fed. Proc.*, **30**, 670(1971).

(4) R. D. Hollifield and J. D. Conklin, *J. Pharm. Sci.*, **62**, 271 (1973).

(5) P. L. Cox, J. P. Heotis, D. Polin, and G. M. Rose, *ibid.*, **58**, 987(1969).

ACKNOWLEDGMENTS AND ADDRESSES

Received October 16, 1972, from the *Unit of Drug Distribution, Eaton Laboratories, Norwich Pharmacal Company, Norwich, NY 13815*

Accepted for publication January 24, 1973.

▲ To whom inquiries should be directed.

COMMUNICATIONS

Dansyl Derivatives of Δ^9- and Δ^8-Tetrahydrocannabinols

Keyphrases □ Dansyl derivatives of Δ^9- and Δ^8-tetrahydrocannabinols—synthesis, physical and chemical properties □ Tetrahydrocannabinols—*synthesis of dansyl derivatives*, physical and chemical properties

Sir:

The use of dansyl chloride (5-dimethylamino-1-naphthalenesulfonyl chloride, I) in the fluorometric determination of amines and phenols is well documented (1). Dansylated cannabinol-related compounds were recently derived from blood, urine, and saliva samples following hashish administration (2). As a prerequisite for the development of suitable methodology for the assay of compounds and metabolites derived from *Cannabis sativa*, a series of dansyl derivatives of several cannabinoid compounds was prepared in micromolar quantities and their TLC properties were reported (3). The preparation of larger quantities of dansyl-Δ^9-tetrahydrocannabinol (II) and dansyl-Δ^8-tetrahydrocannabinol (III) was accomplished to determine the physical and chemical properties of these derivatives.

The reaction of Δ^9- or Δ^8-tetrahydrocannabinol and excess dansyl chloride was performed in acetone–water solution saturated with sodium carbonate at 40° for 2 hr. After excess dansyl chloride was hydrolyzed by treatment with base, the reaction mixture was extracted with ethyl acetate. Compounds II and III were isolated as thick oils. Crystallization could only be induced by prolonged storage at −5° in hexane. Recrystallization from hexane or heptane yielded crystalline solids which provided microanalytical values within acceptable limits and the correct molecular weight by mass spectrometry. The physical and spectral characteristics of Compounds II and III are summarized in Tables I and II.

The reaction of I would be expected to occur on the phenolic group of tetrahydrocannabinol. Examples are known with reactions similar to dansylation where the substituent does not add onto the phenolic oxygen but

Table I—Physical and Spectral Characteristics of Dansyl-Δ^9- and Dansyl-Δ^8-tetrahydrocannabinols (II and III)

	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 Δ^9- or Δ^8-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.

Table II—Low-Resolution Mass Spectra of Dansyl-Δ^9- and Dansyl-Δ^8-tetrahydrocannabinols (II and III)

	II			III	
	m/e	Relative Abundance		*m/e*	Relative Abundance
M^+	547	8	M^+	547	18
$M-SO_2$	483	3	$M-SO_2$	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			

(1) N. Seiler and M. Wiechmann, in "Progress in TLC and Related Methods," vol. I, A. Niederwieser and G. Pataki, Eds., Ann Arbor-Humphrey, Ann Arbor, Mich., 1970, pp. 94–144.

(2) W. W. Just, G. Werner, and M. Wiechmann, *Naturwissenschaften*, **59**, 222(1972).

(3) I. S. Forrest, D. E. Green, S. D. Rose, G. C. Skinner, and D. M. Torres, *Res. Commun. Chem. Pathol. Pharmacol.*, **2**, 787 (1971).

(4) Y. Gaoni and R. Mechoulam, *J. Amer. Chem. Soc.*, **93**, 217 (1971).

(5) K. Bailey and D. Verner, *Chem. Commun.*, **1972**, 89.

(6) J. F. Lawrence and R. W. Frei, *J. Chromatogr.*, **66**, 93(1972).

ARMEN P. MELIKIAN▲
IRENE S. FORREST
Department of Psychiatry
Stanford University School of Medicine
Stanford, CA 94305
and
Biochemical Research Laboratory
Veterans Administration Hospital
Palo Alto, CA 94304

Received January 19, 1973.
Accepted for publication March 15, 1973.
Supported by Contract HSM-42-71-100 from the National Institute of Mental Health.

▲ To whom inquiries should be directed.

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-