Assuming the single fatty acid residue to be bound to one of the amino groups and the presence of a single free carboxyl group as indicated by the titration curve,[11] the amino acids given above can be joined to give an empirical formula $C_{56}H_{99}O_{14}N_{16}$. This corresponds to a molecular weight of 1220. Analytical data obtained on the hydrochloride are in agreement with this formula.

Optical rotations were taken on the residues obtained by evaporation of the solvents directly from the distribution shown in Fig. 4 and again after recrystallizing the residues. In the case of leucine and phenylalanine no change in rotation was noted after crystallization. The phenylalanine was of the D-configuration. With threonine the rotation of the residue was lower than expected and indicated a slight degree of racemization during hydrolysis. The exact amount is somewhat uncertain because the chloride content of the residue was not determined. When converted to the free amino acid and crystallized, the full rotation was noted.

In the case of α,γ-diaminobutyric acid the rotation of the residue obtained directly from the distribution indicated nearly one third to be racemic, or perhaps one of the six residues to be of the D-configuration. After recrystallization the rotation was only very little higher and still roughly two thirds of that of the L form.

The next step in the study of the structure of this peptide will involve partial hydrolysis in order to determine the sequence of the amino acids. This is being undertaken.

The authors wish to thank Dr. H. Jaffe for his assistance in the spectral studies. We wish to acknowledge with appreciation the technical assistance of Miss E. A. Jacobs and Miss G. Walker. The elementary analyses were carried out by Mr. D. Rigakos and Mr. T. Bella.

NEW YORK, N. Y.

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[CONTRIBUTION FROM THE LABORATORY OF CHEMICAL PHARMACOLOGY, NATIONAL CANCER INSTITUTE[1]]

The Structure of Savinin

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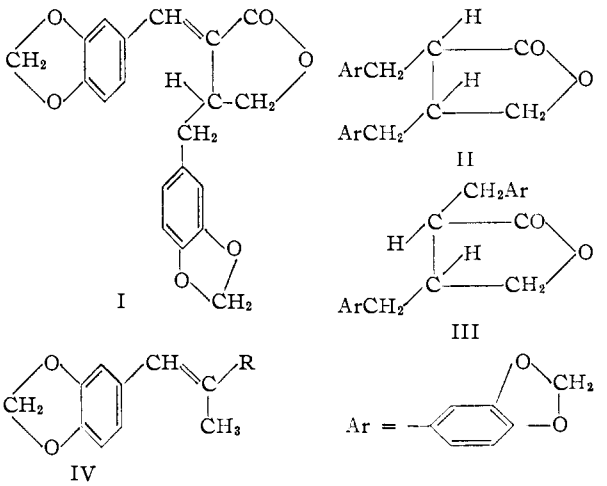
The structure of savinin (I) has been established by its hydrogenation to (+)-isohinokinin (II) and by spectroscopic evidence of its α,β-unsaturated lactone nature. A pronounced bathochromic effect in the ultraviolet spectra of lactones of the savinin type is discussed.

In addition to podophyllotoxin, a compound devoid of tumor-damaging potency was isolated from the dried needles of *Juniperus sabina* and named savinin.[2] Although it had been recrystallized to a constant melting point of 146–148°, the presence of some contaminant was indicated by its analysis.[2] Further purification by chromatography, followed by additional recrystallizations and prolonged drying *in vacuo* has now provided material which still melted at 146.2–147.3°, but now gave analytical figures agreeing closely with the empirical formula $C_{20}H_{16}O_6$. Catalytic hydrogenation afforded dihydrosavinin, $C_{20}H_{18}O_6$, which appeared to be identical with (+)-isohinokinin[3] (II) by its optical rotation and melting point.[4] This was established by base-catalyzed epimerization to (−)-hinokinin[3,5] (III), the identity of which was proved by mixed melting point determination and comparison of infrared spectra.[6]

Several structural formulas for savinin are consistent with its optical activity and its hydrogenation to (+)-isohinokinin. A choice can be made on the basis of the ultraviolet and infrared spectra, which demonstrate that savinin must possess the α,β-unsaturated lactone structure I and exclude alternative β,γ-unsaturated lactone formulations. Thus the ultraviolet absorption spectrum of savinin is characteristic of that of 3,4-methylenedioxycinnamic acid derivatives.[7,8] This is illustrated by comparison of its spectrum with those of methyl α-methyl-3,4-methylenedioxycinnamate (IV, R = $-CO_2CH_3$) and of α-methyl-3,4-methylenedioxycinnamyl acetate (IV, R = $-CH_2OCOCH_3$) (Fig. 1). The infrared spectra (Fig. 2) of both savinin



(1) National Institutes of Health, Public Health Service, U. S. Department of Health, Education and Welfare.

(2) J. L. Hartwell, J. M. Johnson, D. B. Fitzgerald and M. Belkin, THIS JOURNAL, **75**, 235 (1953).

(3) S. Keimatsu and T. Ishiguro, *J. Pharm. Soc. Japan*, **56**, 103, 901 (1936) (German summaries: pp. 19, 187).

(4) Dihydrosavinin was first obtained in the form of needles, m.p. 106–107°, unchanged after recrystallization, while subsequent experiments provided leaflets, m.p. 116.3–116.7° (lit.[3] plates, m.p. 116–117°). Chloroform solutions of these polymorphic modifications had identical infrared spectra and optical rotations.

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(6) We are indebted to Prof. R. D. Haworth for an authentic sample of (−)-hinokinin.

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and the cinnamic acid ester IV (R = $-CO_2CH_3$) show a pronounced C=C stretching vibration between 1640 and 1650 cm.$^{-1}$, while the cinnamyl acetate IV (R = $-CH_2OCOCH_3$) has only a weak band at *ca.* 1670 cm.$^{-1}$. This is consistent with the known fact[9,10] that the ethylenic band is much stronger in α,β-unsaturated carbonyl compounds than in analogous unconjugated compounds. The shift of the carbonyl band toward lower frequencies (from 1770 cm.$^{-1}$ in hinokinin and isohinokinin to 1750 cm.$^{-1}$ in savinin) is also characteristic of α,β-unsaturation.[11]

The configuration at the carbon atom carrying the hydroxymethyl group must be the same in savinin (I) as in (−)-hinokinin (III) since this carbon atom is not involved in the hydrogenation leading to (+)-isohinokinin (II). Postulating *syn* addition in catalytic hydrogenation,[12] the formation of (+)-isohinokinin rather than (−)-hinokinin is consistent with the evidence[5,13] that the former possesses a *cis*-(II) and the latter a *trans* configuration (III).

In connection with the ultraviolet spectra (Fig. 1), a further observation seems of interest. It was suggested in a preceding paper[14] that the bathochromic shift which accompanies lactonization of γ-apopodophyllic acid (max. 327 mμ) to γ-apopicropodophyllin (max. 350 mμ) was caused by the greater conjugation effect of an ester group as contrasted with that of a carboxylic acid group. This suggestion must be revised in the light of evidence obtained both from the literature and from the present work. Comparison of the ultraviolet spectra of α,β-unsaturated acids and of their esters[8,15] indicates that if any bathochromic shift occurs at all, it is of the order of 1–3 mμ only. Thus the maximum at 313 mμ in the acid IV (R = $-CO_2H$) is displaced to 315.5 mμ in its methyl ester. On the other hand, the hydroxy acid corresponding to I (savinic acid) has an inflection at 307.5 mμ (resulting from a lowered extinction coefficient and consequent damping of the maximum by the neighboring band, max. 287.5 mμ), while lactonization shifts the maximum to 334 mμ in I. The pronounced bathochromic shift is, therefore, not caused by esterification, but by the presence of the lactone ring. Both savinin and γ-apopicropodophyllin are α,β-unsaturated γ-lactones with an exocyclic double bond. We were unable to find comparative data on other compounds of this type in the literature. In α,β-unsaturated lactones with an endocyclic double bond, analogous bathochromic effects are much smaller if they occur at all. Thus the absorption maxima of senecioic acid (V, R = CH_3) and its ethyl ester,[15] as well as of steroid 17-$\Delta^{\alpha,\beta}$-butenolides (VI, R = cycloalkyl)[16] are all located

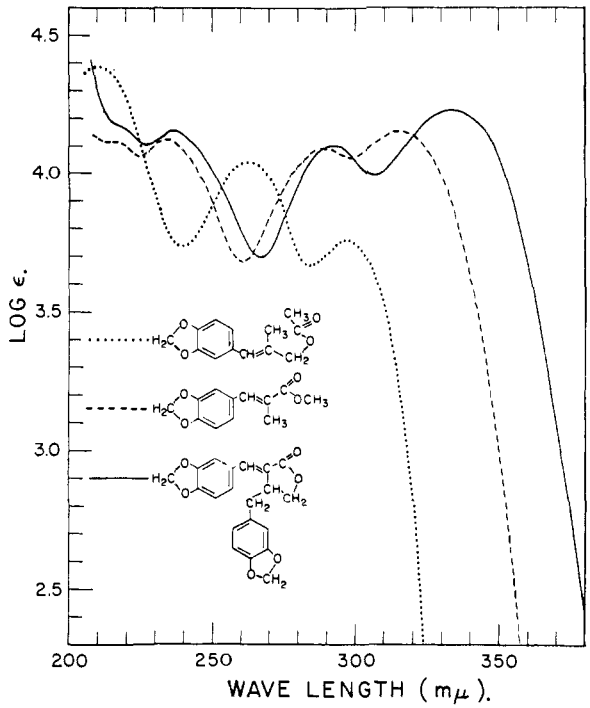
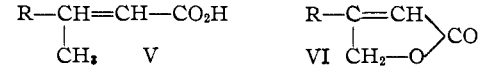


Fig. 1.—Ultraviolet absorption spectra in 95% ethanol of: ——, savinin (I); -----, methyl α-methyl-3,4-methylenedioxycinnamate (IV, R = $-CO_2CO_3$); ...., α-methyl-3,4-methylenedioxycinnamyl acetate (IV, R = $-CH_2OCOCH_3$).

at 217 mμ. The maximum is displaced from 263 mμ in β-methylcinnamic acid (V, R = C_6H_5)[17] to 273 mμ in β-phenyl-$\Delta^{\alpha,\beta}$-butenolide (VI, R = C_6H_5),[18] and a similar shift (from 290[7] to 302 mμ[18]) is found in the corresponding *p*-methoxyphenyl compounds. Increased coplanarity of the conjugated centers[19] and other factors connected with the relative positions in space of the carbonyl and aryl groups may account for the bathochromic effect that accompanies lactonization. Furthermore, the bathochromic shift in savinin and γ-apopicropodophyllin might be related to increased strain in the exocyclic double bonds,[16,20] although evidence on the latter point is conflicting.[16,19,21]



Experimental[22]

Purification of Savinin (I).—A solution of the previously[2] prepared material (m.p. 146.4–148.4°) in chloroform was chromatographed on alumina and eluted with the same solvent. The eluate was concentrated with addition of ethanol and the product recrystallized once more from chloroform–ethanol, then dried *in vacuo* at 110° for three days;

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(22) Corrected melting points were determined with the Hershberg apparatus. Ultraviolet spectra were measured with a Beckman model DU spectrophotometer in 95% ethanol. Infrared spectra were obtained with a Perkin–Elmer model 21 spectrometer in chloroform.

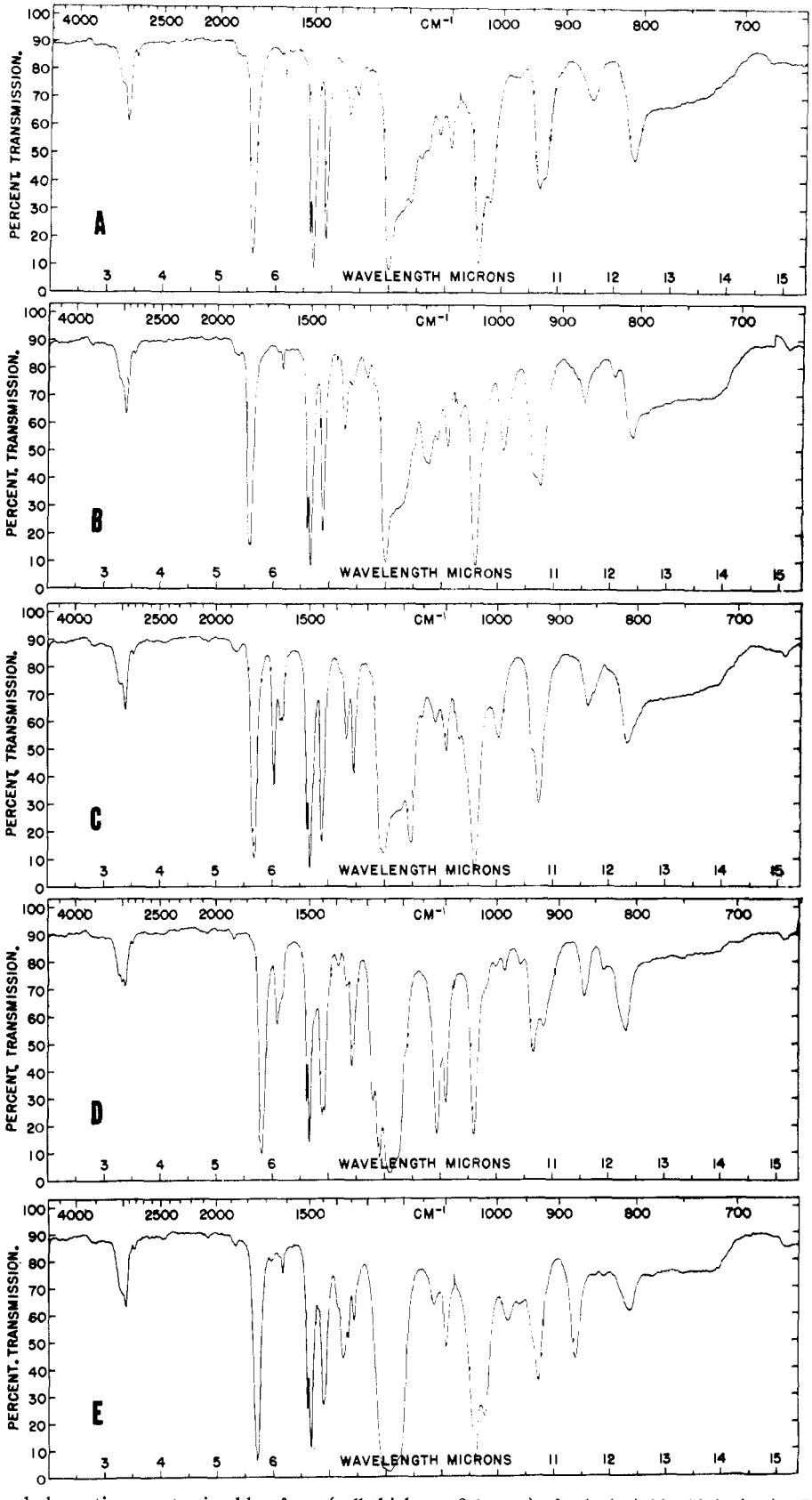

Fig. 2.—Infrared absorption spectra in chloroform (cell thickness 0.1 mm.) of: A, (−)-hinokinin (III); B, (+)-isohinokinin (II); C, savinin (I); D, methyl α-methyl-3,4-methylenedioxycinnamate (IV, R = $-CO_2CH_3$); E, α-methyl-3,4-methylenedioxycinnamyl acetate (IV, R = $-CH_2OCOCH_3$).

m.p. 146.2–147.3°, $[\alpha]^{22}D$ −88° (*c* 1.00, chloroform); λ_{max}^{EtOH} 334, 293, 237 mμ (log ε 4.23, 4.10, 4.16); λ_{min}^{EtOH} 307, 267, 227 mμ (log ε 3.99, 3.70, 4.11).

Anal. Calcd. for $C_{20}H_{16}O_6$: C, 68.18; H, 4.58. Found: C, 68.24; H, 4.71.

Savinic Acid.—Savinin (176 mg.) was boiled with 1.5 ml. of *N* sodium hydroxide and 3 ml. of ethanol for several minutes, and the clear solution diluted with water. On cooling, the sparingly soluble sodium salt precipitated as colorless leaflets. The mixture was chilled in ice, acidified with dilute acetic acid, and the white solid collected, washed with water and dissolved in a small amount of ethanol. The solution was diluted with benzene, then with water and placed in the refrigerator. The product (80 mg.), which was collected after several hours and dried *in vacuo* at room temperature, formed colorless felt-like needles, m.p. 108° (dec., remelting at 145.5–146.5° after resolidification), $[\alpha]^{20}D$ +64° (*c* 0.18, ethanol); λ_{max}^{EtOH} 287.5, 230.5 mμ (log ε 4.08, 4.18); λ_{min}^{EtOH} 258.5, 227 mμ (log ε 3.63, 4.17); λ_{infl}^{EtOH} 307.5 mμ (log ε 3.97).

Anal. Calcd. for $C_{20}H_{18}O_7$: C, 64.86; H, 4.90. Found: C, 64.86; H, 4.66.

(+)-Isohinokinin (Dihydrosavinin) (II).—A suspension of 5.0 g. of savinin and 2 g. of 10% palladium-on-charcoal in 100 ml. of glacial acetic acid was hydrogenated at room temperature and slightly above atmospheric pressure for 40 minutes, at which time the starting material had dissolved and absorption was completed. The catalyst was removed (Celite), washed with hot acetone, and the filtrate evaporated *in vacuo*. Chromatography on alumina, using chloroform, followed by evaporation and crystallization from methanol, yielded 4.63 g. (92%) of colorless leaflets,[4] m.p. 115.8–116.0°. Recrystallization from methanol afforded a product, m.p. 116.3–116.7° (lit.[3] 116–117°), $[\alpha]^{21}D$ +107° (*c* 1.03, chloroform) (lit.[3] +106°).

Anal. Calcd. for $C_{20}H_{18}O_6$: C, 67.79; H, 5.12. Found: C, 67.89; H, 5.20.

(−)-Hinokinin (III).—The previously used procedure[3] was modified in the light of the study of the matairesinol–isomatairesinol equilibrium.[23] A suspension of 4.60 g. of dihydrosavinin in 60 ml. of 3.2% methanolic potassium hydroxide was allowed to stand at room temperature with occasional swirling for 19 hours, during which time the solid dissolved. The solution was acidified with 3 ml. of glacial acetic acid, boiled for 1.5 hours and evaporated to dryness. The residue was treated with 30 ml. of water and 5 g. of sodium carbonate, and the lactone extracted with chloroform, which was then washed with water, dried with magnesium sulfate and evaporated. Crystallization from methanol provided 3.83 g. (83%) of colorless material melting at 55–62°. Recrystallization afforded prisms, m.p. 58–62° (lit. 63–64°,[3] 65–66°[5]), $[\alpha]^{20}D$ −35° (*c* 1.00, chloroform) (lit. −33°,[3] −34°[5]). The m.p. of the product was not depressed by admixture of authentic (−)-hinokinin,[6] and both had identical infrared absorption spectra.

Methyl α-Methyl-3,4-methylenedioxycinnamate (IV, R = $-CO_2CH_3$).—Ide and Buck's procedure for the preparation of the corresponding acid[24] was modified since it has been described as unsafe by the authors. To a stirred suspension of 6.05 g. of sodium hydride[25] in 77 ml. of methyl propionate was added, with cooling in an ice-bath, 0.3 ml. of absolute ethanol, then dropwise over 30 minutes a mixture of 30 g. of piperonal and 20 ml. of methyl propionate. The ice-bath was removed 30 minutes later and stirring continued for another 30 minutes, with occasional immersion in ice-water to control the temperature rise. Glacial acetic acid (18 ml.) was then added cautiously, followed by 20 ml. of water. The organic layer was separated, washed with 2 *N* hydrochloric acid and aqueous potassium carbonate, dried with potassium carbonate and evaporated. Crystallization from methanol yielded 28.9 g. (66%) of pale yellow plates, m.p. 75.5–77.5°. From the mother liquor there was obtained, after saponification and crystallization from ethanol, 2.7 g. (6.5%) of the corresponding acid, m.p. 197–200°. The crude ester was purified by chromatography on alumina, elution with chloroform and recrystallization from methanol, providing colorless glistening plates, m.p. 76.3–77.0°; λ_{max}^{EtOH} 315.5, 290, 234, 217 mμ (log ε 4.15, 4.09, 4.13, 4.11); λ_{min}^{EtOH} 299, 260.5, 225, 214 mμ (log ε 4.05, 3.68, 4.06, 4.11).

Anal. Calcd. for $C_{12}H_{12}O_4$: C, 65.44; H, 5.49. Found: C, 65.28; H, 5.58.

α-Methyl-3,4-methylenedioxycinnamic acid (IV, R = $-CO_2H$) was prepared in quantitative yield by saponification of the pure methyl ester with boiling methanolic potassium hydroxide, followed by concentration and acidification. It crystallized from ethanol in colorless prisms, m.p. 200.3–201.0° (lit.[24] 200°); λ_{max}^{EtOH} 313, 288, 232.5, 218 mμ (log ε 4.13, 4.10, 4.13, 4.13); λ_{min}^{EtOH} 298.5, 258.5, 224, 211.5 mμ (log ε 4.05, 3.70, 4.08, 4.10).

α-Methyl-3,4-methylenedioxycinnamyl Alcohol (IV, R = $-CH_2OH$).—A convenient modification of procedures used for preparing analogous cinnamyl alcohols[26] was employed. A solution of 4.40 g. (0.02 mole) of the methyl ester IV (R = $-CO_2CH_3$) in 200 ml. of dry ether was stirred magnetically and cooled in a Dry Ice–ethanol-bath, while 0.012 mole of lithium aluminum hydride in 25 ml. of dry ether was added dropwise over ten minutes. The temperature was allowed to rise, with continued stirring, to 10° over 30 minutes, during which time a white solid separated. Decomposition was effected by adding 3 ml. of ethyl acetate, then 3 ml. of saturated ammonium chloride solution. The precipitate was filtered off, washed with ether, treated with additional ammonium chloride solution, and the suspension extracted with more ether. The combined ether solutions were washed with water, dried with magnesium sulfate and concentrated to *ca.* 15 ml. Addition of 120 ml. of hexane precipitated a colorless oil which crystallized on chilling and scratching; yield 2.72 g. (71%), m.p. 44.4–45.9°. Recrystallization from ether–hexane provided colorless prismatic needles, m.p. 45.0–45.8°; λ_{max}^{EtOH} 297, 261, 210 mμ (log ε 3.72, 4.05, 4.40); λ_{min}^{EtOH} 283.5, 238 mμ (log ε 3.60, 3.75).

Anal. Calcd. for $C_{11}H_{12}O_3$: C, 68.73; H, 6.29. Found: C, 68.57; H, 6.26.

α-Methyl-3,4-methylenedioxycinnamyl Acetate (IV, R = $-CH_2OCOCH_3$).—A solution of 1.01 g. of IV (R = $-CH_2OH$) in 5 ml. of dry pyridine and 7.5 ml. of acetic anhydride was kept at room temperature for three days, decomposed with ice and extracted with ether. The ether solution was washed with ice-cold dilute hydrochloric acid, sodium bicarbonate solution and water, then dried with magnesium sulfate. Evaporation of solvent and vacuum distillation yielded 0.73 g. of colorless liquid, b.p. 146° (0.6 mm.), $n^{21}D$ 1.5557; λ_{max}^{EtOH} 297, 263, 210.5 mμ (log ε 3.76, 4.04, 4.39); λ_{min}^{EtOH} 284, 240 mμ (log ε 3.67, 3.74).

Anal. Calcd. for $C_{13}H_{14}O_4$: C, 66.65; H, 6.02. Found: C, 66.63; H, 6.31.

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