Identification of Products. Most reaction product mixtures were analyzed on a Finnegan Model 3000 GC peak identifier with a quadrupole mass filter. Mass spectra were obtained at 70 eV for the reaction products eluted from a 6 ft × 0.125 in. stainless steel, 3% OV-1/Chromosorb W column.

GC analyses for the reaction mixtures were made on two dual column instruments (Hewlett-Packard Models 5700A and 5830A) equipped with hydrogen flame ionization detectors. The following 0.125-in. stainless steel columns were used: 2% OV-17/Chromosorb W, 3% UCW-982/Chromosorb W, and 3% OV-225/Chromosorb W.

For all of the systems studied, comparisons between the relative retention times of reaction products and authentic materials on two different columns, and noting peak enhancement upon "spiking" reaction mixtures with authentics comprised one approach for product identification. The mass spectra were used to determine the identities of many of the products (comparison to mass spectra of authentic available products was performed where possible). In this manner 3- and 4-nitro-*o*-xylene and all the benzoic acids and dimethylphenyl benzoates (Table VII) were identified.

o-Methylbenzyl nitrate was determined on the basis of its mass spectrum (molecular ion at *m/e* 167, base peak at *m/e* 91). The product identified as *o*-xylene dimers consisted of three closely spaced peaks all with similar mass spectra (molecular ion at *m/e* 210, base peak at *m/e* 77). None had the exact retention time of 2,2′-dimethylbibenzyl (mp 57–58 °C, mass spectrum molecular ion at *m/e* 210, base peak at *m/e* 105) synthesized by refluxing di-*tert*-butyl peroxide in excess *o*-xylene and removing unreacted monomer by distillation under reduced pressure.

To demonstrate unequivocally that the aroyloxy groups were attached to *o*-xylene at a nuclear position rather than at a side chain in the aryl ester products a concentrated product mixture from a *p*-methylbenzoyl nitrate–*o*-xylene run was hydrolyzed with 5% methanolic potassium hydroxide. The basic aqueous extract of the resulting mixture yielded *p*-methylbenzoic acid and 3,4-xylenol upon acidification (GC analysis), while the original organic portion no longer contained any aryl ester product or any new alcohol product.

All of the hemimellitene products were identified on the basis of their mass spectra and relative GC retention times. 2,3,4-Trimethylphenyl benzoate (mass spectrum molecular ion at *m/e* 240, base peak at *m/e* 105) eluted before 3,4,5-trimethylphenyl benzoate (mass spectrum molecular ion at *m/e* 240, base peak at *m/e* 105). Other products included a chlorohemimellitene (mass spectrum molecular ion at *m/e* 154, M + 2 at *m/e* 156) (structure not determined, but likely an α-chloro isomer[13]) from the cupric chloride–benzoyl peroxide reaction and 4-nitrohemimellitene (mass spectrum molecular ion at *m/e* 165, base peak at *m/e* 119) and 5-nitrohemimellitene (mass spectrum molecular ion at *m/e* 165, base peak at *m/e* 119) from both the benzoyl nitrate and benzoyl peroxide–nitric acid reactions. Also observed from this latter system were a dimethylbenzaldehyde isomer (mass spectrum molecular ion at *m/e* 134), a dimethylbenzyl alcohol isomer (mass spectrum molecular ion at *m/e* 136) and a trimethylbiphenyl isomer (mass spectrum molecular ion at *m/e* 196).

Reaction product yields were determined using phenyl benzoate or carbazole as an internal standard, and comparing product areas to that of the marker added in known amount. The mean of duplicate analyses in good agreement was used, and response factors were calculated for mixtures of authentic vs. the internal standard and used to correct area ratios to mole ratios. The benzoic acid by-product yields were determined in two cases (Table I) by aqueous base extraction and isolation. Together with the aryl ester products in these two runs the benzoyloxy moiety material balance from the *p*-nitrobenzoyl nitrate and benzoyl nitrate reactions was 95 and 86%, respectively.

Registry No.—$AgNO_3$, 7761-88-8; *p*-methoxybenzyl chloride, 100-07-2; *p*-methylbenzoyl chloride, 874-60-2; benzoyl chloride, 98-88-4; *p*-chlorobenzoyl chloride, 122-01-0; *p*-fluorobenzoyl chloride, 403-43-0; *p*-nitrobenzoyl chloride, 122-04-3; *o*-xylene, 95-47-6; hemimellitene, 526-73-8.

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Biosynthetic Oxidation and Rearrangement of Vittatine and Its Derivatives[1]

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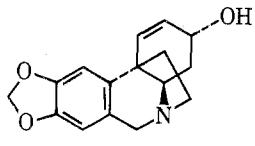
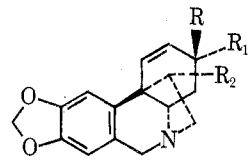
By the use of appropriately labeled radioactive precursors it is demonstrated that vittatine (**2d**) is a precursor of haemanthamine (**2e**) and montanine (**4a**) in *Rhodophiala bifida*. Evidence is presented to show there is no equilibration of the enantiomeric (−)- and (+)-crinine ring systems in the plants.

Although the Amaryllidaceae alkaloids are almost invariably isolated in optically active form, the family is unique because a given plant may produce two very similar alkaloids possessing enantiomeric nuclei. Thus *Nerine bowdenii* W. Wats. contains both crinine (**1**) and (+)-epicrinine (**2a**).[2,3] Several plants contain the optical antipode of **1**, vittatine (**2d**),[4–9] but racemic crinine has never been isolated. Because more oxygenated derivatives of **1** and **2a** have been



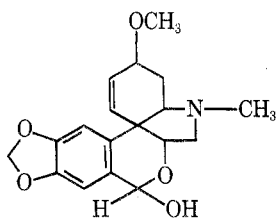
1



2

a, R_1 = OH; R, R_2 = H
b, R = H; R_1 = OCH_3; R_2 = OH
c, R, R_1 = O; R_2 = H
d, R = OH; R_1, R_2 = H
e, R = OCH_3; R_1 = H; R_2 = OH
f, R = OH, R_1 = H; R_2 = OH
g, R = OCH_3; R_1, R_2 = H

converted either chemically or in vivo into other ring systems (e.g., **3**, **4**, and **5**) it was of interest to follow the pathways of **1** and **2a** in the plant to other transformation products.



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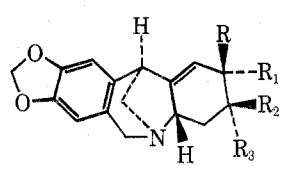
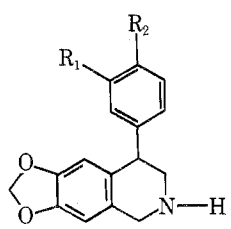


4

a, R = H; R_1 = OCH_3; R_2 = OH; R_3 = H
b, R = OCH_3; R_1 = H; R_2 = OH; R_3 = H
c, R = H; R_1 = OCH_3; R_2 = OCH_3; R_3 = H
d, R = H; R_1 = OH; R_2 = OH; R_3 = H



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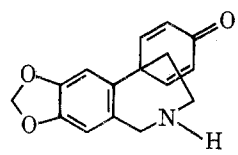


6

It was of biosynthetic importance to determine whether **1** and **2** (and similar alkaloids) could be interrelated in vivo, perhaps through conversion to the symmetrical dienone (**6**) as an intermediate. (−)-Crinine (**1**) labeled with tritium in the aromatic ring was fed to *Nerine bowdenii*. After 3 weeks of plant growth, the alkaloids were isolated in the usual manner. All alkaloids derived from the (−)-crinine nucleus were radioactive while the crinamine (**2b**) and (+)-epicrinine (**2a**) were inactive. While this preliminary experiment indicated that **1** is not converted to the ring system of **2** in the plant, oxovittatine (**2c**) might be a more likely precursor of **6** since only β-elimination of the amino function would be required. It could also be rationalized that oxidation of **2a** to **2c** might, for some reason, be unusually difficult in the plant. The requisite [^{3}H]oxovittatine was readily prepared by the manganese dioxide oxidation of *ar*-[^{3}H]vittatine. Further oxidation to *N*-ethylhydrastimide showed that 96% of the label was located in the aromatic ring. An aqueous solution of the tracer was introduced by syringe into eight bulbs of *N. bowdenii*. The alkaloids were isolated after 3 weeks of additional growth in a greenhouse. As expected, lycorine and belladine were inactive. Consistent with previous findings, all alkaloids derived from the nucleus of **1** were nonradioactive. Both crinamine (**2b**) and (+)-epicrinine (**2a**) were radioactive (Table I) but the low percentage of incorporation leaves doubt whether oxovittatine is on the main biosynthetic route to the crinine-type alkaloids. The radioactive crinamine was degraded to *N*-ethylhydrastimide which contained, within experimental error, the same specific activity as the radioactive precursor. From the results of these two tracer studies we conclude that there is no evidence that the enantiomeric rings systems of **1** and **2** are interconvertible in the plant.

Table I. Incorporation of [^{3}H]Oxovittatine in *N. bowdenii*[a]

Alkaloid	Activity, dpm/mM	Dilution	% inc.
Crinamine	4.64×10^6	3.76×10^3	0.06
(+)-Epicrinine	6.59×10^6	2.63×10^3	0.09

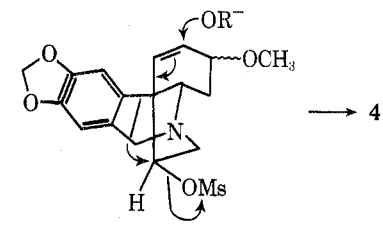
[a] The total activity of the [^{3}H]oxovittatine was 0.88 mCi.

Table II. Incorporation of *ar*-[^{3}H]Vittatine in *R. bifida*[a]

Alkaloid	Activity, dpm/mM	Dilution	% inc.
Haemanthamine	35.2×10^6	2.8×10^3	0.20
Montanine	4.21×10^6	23.4×10^3	0.06

[a] 1.64 mCi of [^{3}H]vittatine was introduced.

The alkaloids montanine, coccinine, manthine, and pancracine are based on the 5,11-methanomorphanthridine nucleus (**4**). The structures assigned to the individual alkaloids were determined by degradation and by partial syntheses which involved the rearrangement of **2b** or **2e** as the



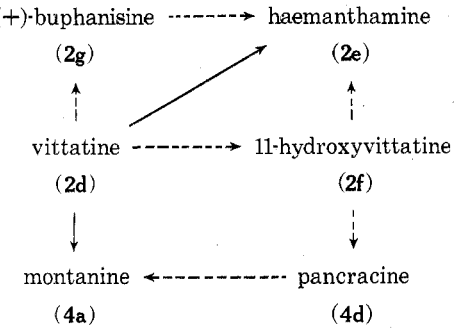
2b or **2e**

mesylate to the ring system of **4**.[10] In an early attempt to mimic the rearrangement in vivo, *ar*-[^{3}H]haemanthamine was prepared and fed to blooming *Haemanthus coccineus*. This plant is known to contain haemanthamine (**2e**), montanine (**4a**), coccinine (**4b**), and manthine (**4c**).[11] The plants were processed after 1 month. No alkaloid related to **4** was found to be radioactive. The C_3 methoxyl group of haemanthamine would appear to be a point of interference in the in vivo rearrangement since **4a** and **4b** have hydroxyl groups at the equivalent position.

With a supply of *ar*-[^{3}H]vittatine on hand the experiment was repeated with this precursor using *Rhodophiala bifida* which has been reported to contain haemanthamine, vittatine, 11-hydroxyvittatine (**2f**), and montanine (**4a**), among other alkaloids.[12,13] The results given in Table II show that vittatine is a precursor of both haemanthamine and montanine. Both montanine and haemanthamine were converted to *N*-ethylhydrastimide. The specific activities of the imides were 97 and 96%, respectively, of that found for the isolated alkaloids.

With these results several biosynthetic pathways can be considered for the late-stage elaboration of haemanthamine and montanine (Scheme I). Facts and possible bio-

Scheme I. Proposed Montanine Biosynthesis



synthetic routes are designated by solid and dashed arrows, respectively. No biosynthetic experiments have been published to establish the nature of the 5,10b-ethanophenanthridine intermediate, unoxidized at C_{11} (8), which leads to haemanthamine (**2e**). Thus, **2e** could be derived either from vittatine by C_{11} hydroxylation followed by C_3 O-methylation or by the reverse process via (+)-buphanisine (**2g**). Since the latter alkaloid has not been reported to occur in the Amaryllidaceae, and **2f** and **4d** are known to occur in *R. bifida*, the former route must be considered most likely. We suggest that 11-hydroxyvittatine is the common precursor of both **2e** and **4a**.

The specific activity of vittatine-derived haemanthamine is substantially higher than that of the montanine isolated from the same experiment. This finding seems to indicate that 11-hydroxyvittatine is converted more efficiently to haemanthamine which only requires the methylation of the hydroxyl function at C_3 of 11-hydroxyvittatine while the formation of montanine involves a rearrangement of the vittatine ring system in addition to the methylation of the oxygen function at C_2.

Experimental Section

Melting points were taken on a Köfler microscope hot stage apparatus and are corrected. Infrared spectra were taken on either a Beckman Model IR-12 or IR-18A recording spectrophotometer in chloroform solution or as a potassium bromide pellet. The proton magnetic resonance spectra were obtained on a Varian A-60 or HA-100 in chloroform-d_1 unless another solvent was indicated. Mass spectra were recorded on an MS-902 mass spectrometer. This spectrometer was purchased on NSF Grant GP 10226. The measurements of radioactivity were made with a Packard Tri-Carb liquid scintillation spectrometer (Model 3002) at ambient temperature. Solutions for counting were either toluene–PPO–POPOP [4.9 g of 2,5-diphenyloxazole (PPO) and 0.1 g of 1,4-bis-2-(5-phenyloxazole) benzene (POPOP) in 1 l. of dry toluene] or Bray's solution.[14] Efficiency of counting tritium was determined for each sample by means of an internal standard of [^{3}H]- or [^{14}C]toluene. Reproducibility of the assays was ±3%.

Preparative-scale layer chromatography used 20 × 20 cm glass plates coated with silica gel (Merck PF 254 + 366) 0.5 mm in thickness. The plates were eluted once with the solvent system specified. The alkaloids were detected by ultraviolet light. The bands of silica gel and alkaloid were removed from the plate and covered with 80–100 ml of 10% aqueous ammonia and the aqueous layer including the silica gel was extracted with chloroform until the aqueous layer gave a negative silico-tungstic acid test. The alkaloids were identified by their TLC behavior, melting point, mixture melting point, and by comparison of their ir spectra with known reference spectra. The alkaloids were purified from chromatographic fractions via recrystallization from appropriate solvents to constant activity. The percent incorporation was calculated as (100 × total activity of isolated alkaloid) divided by (total activity fed). For this purpose, the final constant activity of the alkaloid per milligram was multiplied by the quantity of alkaloid isolated (mg) which was of good chemical purity as determined by TLC, melting point, and ir.

[^{3}H]Vittatine. *O*-Acetylvittatine (50 mg) was tritiated by the catalytic exchange method using aqueous acetic acid containing 10 Ci of tritiated water and 25 mg of prereduced platinum catalyst at 100 °C. The catalyst was removed and the solution was divided into seven parts. One portion was evaporated under reduced pressure. The residue was dissolved in 15 ml of methanol containing 1 ml of 50% sodium hydroxide. After 2 days at 20 °C, the methanol was removed under reduced pressure and the residue was dissolved in 50 ml of water. The aqueous solution was diluted with 35 mg of inactive vittatine and extracted with chloroform. The chloroform solution was washed repeatedly with water in order to remove the remaining labile tritium. Concentration of the dried chloroform extract gave 30 mg of crude tritiated vittatine. The vittatine was recrystallized from acetone to constant activity to give 10 mg, mp 210–211 °C (lit.[15] mp 207–208 °C), 9.89×10^{10} dpm/mM. Further dilution with nonradioactive alkaloid gave 800 mg (7.29×10^7 dpm/mM) of vittatine which was converted to 2 mg of *N*-ethylhydrastimide,[16] mp 170–171 °C (7.01×10^7 dpm/mM).

[^{3}H]-(−)-Crinine was prepared similarly.

[^{3}H]Oxovittatine. A chloroform solution of 57 mg of [^{3}H]vittatine (1.73×10^{10} dpm/mM) was oxidized with 350 mg of manganese dioxide. The pure ketone (40 mg, mp 185–187 °C, 1.75×10^{10} dpm/mM) was obtained by recrystallization from ether–chloroform.

Incorporation of [^{3}H]Oxovittatine in *N. bowdenii*. An aqueous solution of 30 mg (0.88 mCi) of [^{3}H]oxovittatine (pH 6) was injected into eight growing bulbs. After 3 weeks of growth in a greenhouse, the bulbs (4639 g) were processed for alkaloids.[2] The alkaloid fraction containing chloroform-soluble hydrochlorides (600 mg) was chromatographed on Florisil to give 110 mg of undulatine (mp 151–152 °C) and 128 mg of belladine (as the HCl salt, mp 193–194 °C); both were nonradioactive. The alkaloids forming chloroform-insoluble hydrochlorides (600 mg) afforded 50 mg of inactive lycorine by direct crystallization. The remainder was chromatographed on Florisil to give nonradioactive ambelline, crinamidine, crinine, belladine, and undulatine. The filtrate from the fractions providing ambelline was diluted with 70 mg of crinamine and recrystallized to constant activity, mp 199–200 °C (lit.[17] mp 198–199 °C). The crinine filtrates were diluted with 70 mg of (+)-epicrinine and recrystallized to constant activity, mp 208–209 °C (lit.[17] mp 207–209 °C).

Incorporation of [^{3}H]Vittatine in *R. bifida*. [^{3}H]Vittatine (1.64 mCi, 10 mg) was dissolved in 0.5 ml of water (pH 6) and introduced directly into the *Rhodophiala bifida* bulbs (20 bulbs) with a fine hypodermic needle. The radioactive residue in the vial was dissolved in 0.5 ml of water and injected into six additional bulbs 3 days later. The plants were grown in pots in a greenhouse for 3 weeks after which the bulbs were harvested.

Isolation of Alkaloids from *R. bifida*. The bulbs (437 g) were macerated with 3 l. of 95% ethanol in a Waring Blendor. The mixture was filtered and the filter cake was allowed to stand overnight in 1 l. of 95% ethanol. The solid material was filtered and the filtrates were combined and concentrated under reduced pressure. The resulting residue (5.3 g) was acidified with 2 N hydrochloric acid and filtered to remove the acid-insoluble material. The insoluble material was heated with 2 N hydrochloric acid and refiltered. The filtrates were combined and washed five times with benzene. The benzene extract contained no alkaloidal material and was discarded.

The aqueous acidic solution was made basic (pH 10) with ammonium hydroxide and extracted several times with chloroform. The aqueous solution was then adjusted to pH 12 by the addition of 10% sodium hydroxide and extracted three times with 20% ethanol in chloroform. The organic extracts were combined and concentrated under reduced pressure to give 2.0 g of crude basic material. The basic residue was dissolved in a minimum amount of acetone, and 10 drops of 70% perchloric acid was added followed by enough ether to cause the solution to become turbid. The solution was allowed to stand at 0 °C for 2 h in order to ensure complete precipitation of montanine perchlorate. The product (109 mg) was removed by filtration and recrystallized from acetone to give 100 mg, mp 249–250 °C. The filtrate was dissolved in water, made basic with ammonium hydroxide, and extracted with chloroform. Evaporation of the chloroform gave 1.0 g of a crude residue (1.61×10^6 dpm/mM).

A benzene solution of the crude residue was chromatographed on alumina packed in benzene. Elution with 25% chloroform in benzene afforded fractions from which 62 mg of haemanthamine, mp 199–200 °C, was obtained by crystallization from acetone. Elution with 50–75% chloroform in benzene gave fractions rich in montanine from which 91 mg of montanine perchlorate, mp 250–251 °C, was obtained as before. Further elution with chloroform and 1–10% methanol–chloroform solutions gave no characterizable products.

The haemanthamine and montanine perchlorate were recrystallized to constant activity to give a total of 62 mg of haemanthamine, mp 199–200 °C (lit.[11] 203–203.5 °C, 3.52×10^7 dpm/mM), and 200 mg of montanine perchlorate, mp 250–251 °C (lit.[11] mp 249–250 °C, 4.21×10^6 dpm/mM).

Both alkaloids were diluted with inactive alkaloid for oxidative degradation. Haemanthamine (1.5 g, 1.46×10^5 dpm/mM) gave 50 mg of *N*-ethylhydrastimide, mp 169–170 °C (1.39×10^5 dpm/mM). Oxidation of 1.5 g of montanine (1.31×10^5 dpm/mM) gave 25 mg of imide, mp 169–170 °C (1.27×10^5 dpm/mM).

Registry No.—**1,** 510-67-8; **2a,** 25375-48-8; **2b,** 639-41-8; **2c,** 59013-69-3; **2d,** 510-69-0; **2e,** 466-75-1; **4a** perchlorate, 58944-40-4; **4a** imide, 3990-41-8; *O*-acetylvittatine, 58944-39-1; undulatine, 6882-09-3; belladine HCl, 58944-41-5; ambelline, 3660-62-6.

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Interconversions in the Pluviine–Lycorenine Series[1]

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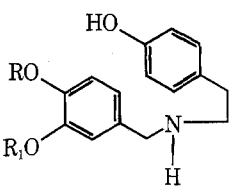
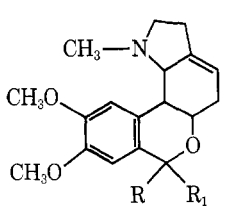
Norpluviine is converted in *Narcissus pseudonarcissus* L. (King Alfred) primarily to alkaloids of the lycorenine type while pluviine undergoes oxidation of the hydroaromatic ring to form unrearranged products in *N. poeticus*. Consistent with our findings on the mechanism of the late stage oxidation of caranine to lycorine, pluviine is converted to galanthine with inversion at C_2. A chemical conversion of lycorine to 7-hydroxylycorine (**12–13**) has been accomplished. Some spectral and chemical properties of the compound are described.

It was recognized in the original biosynthetic hypothesis of oxidative phenyl–phenyl coupling[2] that alkaloids related to lycorenine (**2b**) could not be derived from a norbelladine-type precursor (**1**) directly. The well-documented hemiami-



1

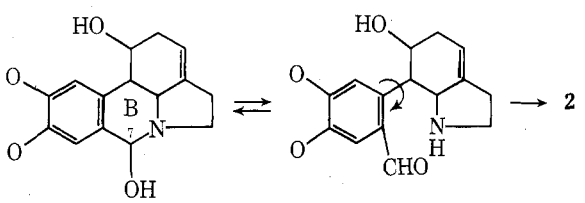
a, R = CH_3; R_1 = H (*O*-methylnorbelladine)
b, R, R_1 = H (norbelladine)



2

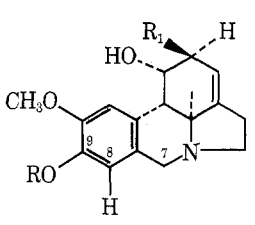
a, R, R_1 = O (homolycorine)
b, R = H, R_1 = OH (lycorenine)

nal–hemiacetal interconversion of haemanthidine and pretazettine[3] suggested that a similar process might occur in alkaloids of the lycorine type.[4] Benzylic oxidation of the CH_2 at C_7 would afford **3**; ring opening to form an amino aldehyde followed by hemiacetal formation and methylation could provide derivatives of **2.**

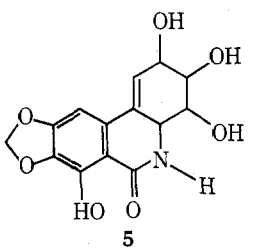


3

To test the validity of this pathway, [8-^{3}H]norpluviine (**4a**) was prepared by tritium exchange. [8-^{3}H]Pluviine (**4b**) was obtained easily by the action of diazomethane on **4a.** The



4



5

a, R = R_1 = H (norpluviine)
b, R = CH_3; R_1 = H (pluviine)
c, R = CH_3; R_1 = OH (methylpseudolycorine)
d, R = CH_3; R_1 = OCH_3 (galanthine)

distribution of the tritium label was determined for radioactive **4b** by oxidation to *m*-hemipinic acid (4,5-dimethoxyphthalic acid) which was converted to the *N*-ethyl imide. The specific molar activity of the imide was 78% of that found for **4b,** indicating that this percentage of the tritium was located in the aromatic ring. An aqueous solution (pH 6) of 5 mg of