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Microbial Metabolism of N-Methylcarbamate Insecticide. II.[1] Synthesis of Metabolites of *o-sec*-Butylphenyl N-Methylcarbamate

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The characterized and anticipated metabolites of *o-sec*-butylphenyl N-methylcarbamate (BPMC) (I) were synthesized. And also it was described about the preliminary experiments with respect to the determination of configurations of *o*-(2-hydroxy-1-methylpropyl) phenyl N-methylcarbamate, main metabolites by *Aspergillus niger* van Tieghem.

In the previous paper,[1] the authors reported on the metabolism of *o-sec*-butylphenyl N-methylcarbamate (BPMC) by *Aspergillus niger* van Tieghem, and several metabolites were isolated and characterized. They were the hydrolyzed, N-demethylated, N-hydroxymethylated, hydroxylated in alkyl side-chain and further oxidized metabolites. The present work deals with the syntheses of these metabolites of BPMC including the anticipated metabolites and also of ^{3}H-BPMC.

N-Demethylated product, *o-sec*-butylphenylcarbamate (III) was prepared by a little modified method of Takeuchi and Ninagawa[3] using *o-sec*-butylphenol (II) and ethylcarbamate.

The treatment of *o*-hydroxyacetophenone (IV) with a Grignard reagent and subsequent hydrolysis yielded *o*-(1-hydroxy-1-methylpropyl)phenol (V), which was relatively labile for preservation and tended to decompose to a product showing a molecular ion peak at *m/e* 296 ($2\times C_{10}H_{14}O_2-2H_2O$) in the mass spectrum even if kept in cold and sealed container. The reaction of V with equimolar methylisocyanate yielded *o*-(1-hydroxy-1-methylpropyl)phenyl N-methylcarbamate (VI).

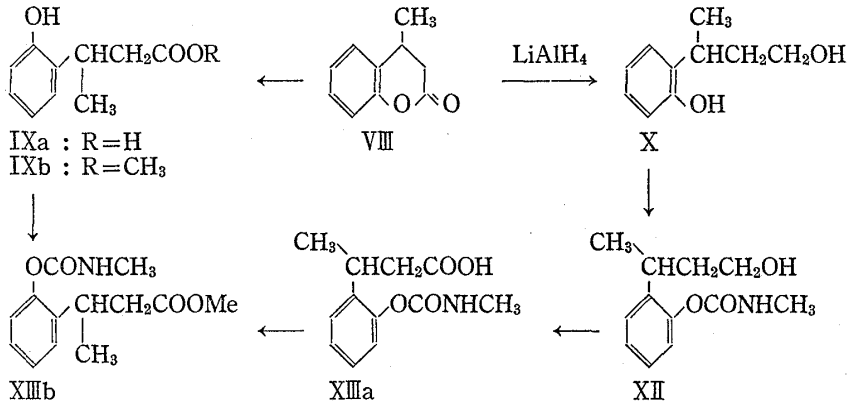

Chart 1

o-(2-Carboxy-1-methylethyl)phenyl N-methylcarbamate (XIIIa) was synthesized as follows. Lithium aluminum hydride ($LiAlH_4$) reduction of 4-methyl-3,4-dihydrocoumarin (VIII)[4] yielded *o*-(3-hydroxy-1-methylpropyl)phenol (X), which was carbamoylated with methylisocyanate to give *o*-(3-hydroxy-1-methylpropyl)phenyl N-methylcarbamate (XII).

1) Part I: T. Suzuki and M. Takeda, *Chem. Pharm. Bull.* (Tokyo), **24**, 1967 (1976).
2) Location: *18-1, Kamiyoga 1-Chome, Setagaya-ku, Tokyo, 158, Japan.*
3) S. Takeuchi and E. Ninagawa, *Bull. Chem. Soc., Jap.* **44**, 3184 (1971).
4) Y. Ito, H. Kitagawa, T. Hiramori, Y. Suzuki, and M. Yamagata, *Yakugaku Zasshi*, **71**, 686 (1951).

On this treatment *o*-(1-methyl-3-N-methylcarbamoyloxypropyl)phenol (XI) was obtained as a by-product. The hydroxy carbamate (XII) was subjected to oxidation to give *o*-(2-carboxy-1-methylethyl)phenyl N-methylcarbamate (XIIIa), which was subsequently converted to a methyl ester (XIIIb). The methyl ester (XIIIb) was also prepared by hydrolysis of the coumarin VIII to make the carboxylic acid (IXa) and esterification, followed by carbamoylation of the resultant phenol ester (IXb). The both compounds were proved completely identical by physicochemical analyses.

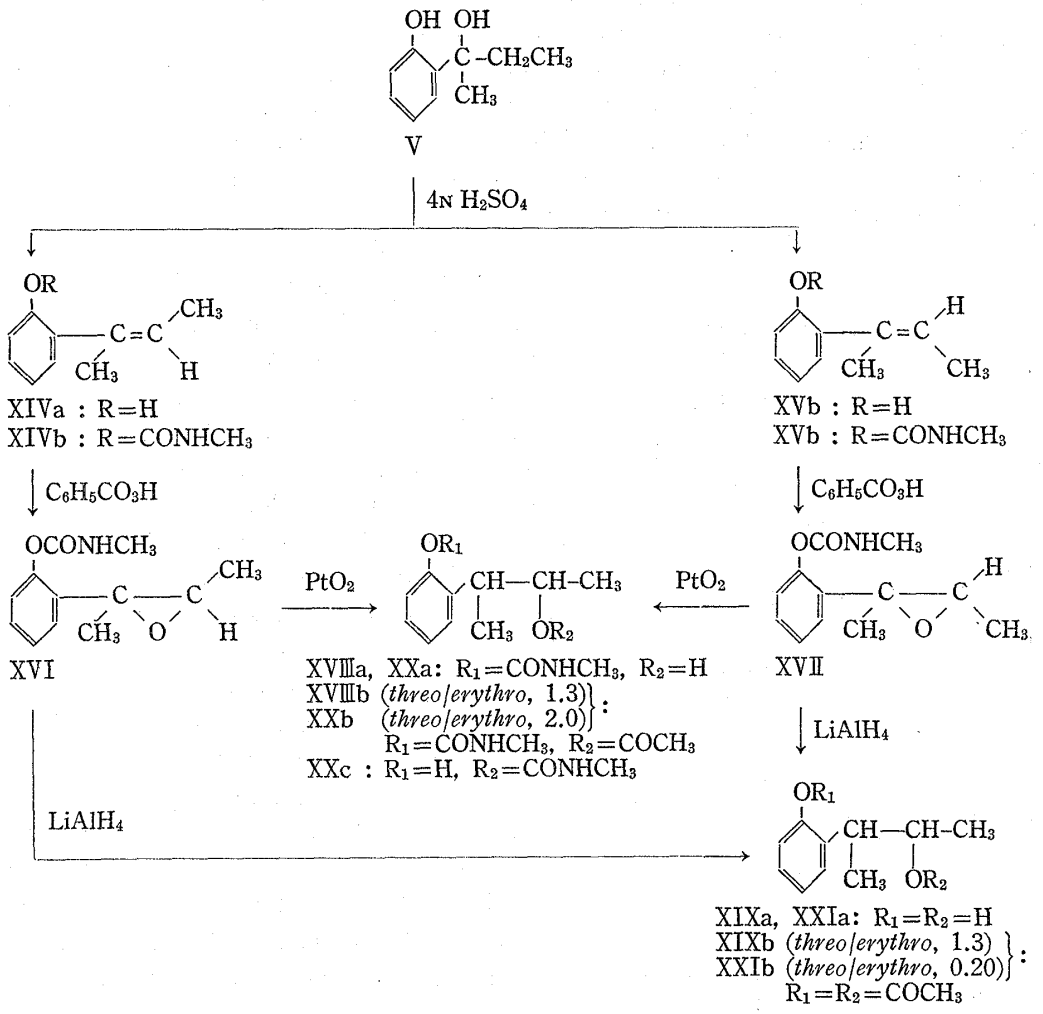

Chart 2

For the purpose of clarifying the configurations of *o*-(2-hydroxy-1-methylpropyl)phenyl N-methylcarbamate, which were obtained as main metabolites of BPMC (I), following reactions were attempted. Namely, V was treated with 4N H_2SO_4 to furnish an approximately equimolar mixture of *Z*-*o*-(1-methyl-1-propenyl)phenol (XVa) and *E*-*o*-(1-methyl-1-propenyl)phenol. (XIVa). Without any further purification, a mixture of XIVa and XVa was subjected to carbamoylation to give a mixture of *Z*-*o*-(1-methyl-1-propenyl)phenyl N-methylcarbamate (XVb) and *E*-*o*-(1-methyl-1-propenyl)phenyl *N*-methylcarbamate (XIVb) which were separated by thin-layer chromatography (TLC).

Reaction of XVb with perbenzoic acid in chloroform afforded *Z*-*o*-(1,2-epoxy-1-methylpropyl)phenyl N-methylcarbamate (XVII), which was then catalytically reduced in acetic acid to yield a mixture of *erythro*- and *threo*-*o*-(2-hydroxy-1-methylpropyl)phenyl N-methylcarbamate (XXa). On these reactions, *o*-(1-methyl-2-N-methylcarbamoyloxypropyl)phenol (XXc) was obtained as an unexpected product, which seemed likely to be derived from XXa by carbamoyl migration in acid catalyzed medium. Such migration of the carbamoyl moiety

may be characteristic of aromatic carbamate compounds, which have a β-hydroxylated alkyl side-chain on *ortho*-position, since we experienced that the reduction of *o*-(1,2-epoxy-1-methylethyl)phenyl N-methylcarbamate[5] over Adams' platinum gave the corresponding migrated compound, *o*-(2-carbamoyloxy-1-methylethyl)phenol, as well. As far as we know, it seems to be a new type migration.

An attempt to obtain *o*-(2-hydroxy-1-methylpropyl)phenyl N-methylcarbamate by equivalent amount of $LiAlH_4$ from XVII resulted in a very poor yield. Treatment of the *Z*-epoxide (XVII) with excess amount of $LiAlH_4$ furnished a mixture of *erythro*- and *thero-o*-(2-hydroxy-1-methylpropyl)phenol (XXIa) in a good yield.

On the other hand, *E-o*-(1,2-epoxy-1-methylpropyl)phenyl N-methylcarbamate (XVI) was catalytically reduced to give a mixture of *erythro*- and *threo-o*-(2-hydroxy-1-methylpropyl)-phenyl N-methylcarbamate (XVIIIa), which was successively acetylated to yield XVIIIb, whereas reaction of XVI with excess amount of $LiAlH_4$ gave also a mixture of *erythro*- and *threo-o*-(2-hydroxy-1-methylpropyl)phenol (XIXa).

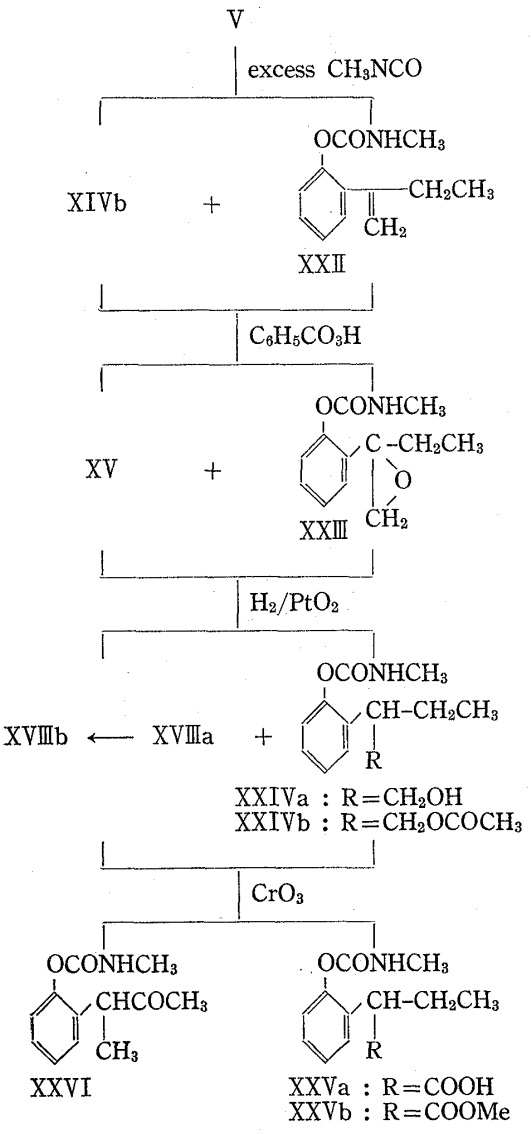

Chart 3

It is well known that reduction of epoxide with $LiAlH_4$ induces trans cleavage.[6] Therefore reduction of *Z*-epoxide with $LiAlH_4$ must give *erythro* and that of *E*-epoxide must afford *threo* isomer. Referring to XVI and XVII, the reduction of *Z*-epoxide (XVII) and *E*-epoxide (XVI) with $LiAlH_4$, followed by acetylation gave a mixture (XXIb and XIXb) of the integral ratios of 0.20 and 1.3 (the signal at 1.92 to that at 2.07 ppm, aliphatic acetoxyls), respectively in the nuclear magnetic resonance (NMR) spectra. Therefore the diacetate which showed the acetoxyl protons at 1.92 ppm should be assigned to a *threo* and the one exhibited those at 2.07 ppm to a *erythro* isomer, respectively.

On the other hand, catalytic reduction of the epoxide (XVII and XVI) on platinum oxide, followed by acetylation gave a mixture (XXb and XVIIIb) of the integral ratios of 2.0 and 1.3 (the signal at 1.95 to that at 2.04 ppm, aliphatic acetoxyls), respectively. The integral ratios of the acetoxyl protons were more than 1 in both XXb and XVIIIb, and the reason, that the integral ratio in XXb exceed to that in XVIIIb, may be attributed to the movement of equilibrium into the more thermodynamically stable *threo* isomer by the stepwise reduction in acid catalyzed medium.

On heating of V under reflux with an excess amount of methylisocyanate in the presence of triethylamine, a mixture of XIVb and *o*-(1-methyleneпropyl)phenyl N-methylcarbamate (XXII) (approx. 3 : 2) was produced (see chart 3). Without further purification, the mixture was

5) Unpublished data.
6) L.W. Trevov and W.G. Brown, *J. Am. Chem. Soc.*, 71, 1675 (1949).

converted into epoxide, reduced catalytically and acetylated to afford XVIIIb and *o*-(1-acetoxymethylpropyl)phenyl N-methylcarbamate (XXIVb). On the other hand, oxidation of a mixture of XXIVa and XVIIIa yielded a mixture of XXVa and *o*-(1-methylacetonyl)phenyl N-methylcarbamate (XXVI).

3-Methyl-2H-1,3-benzoxazine-2,4(3H)-dione (XXVII) was synthesized by the method of Wagner[7] and by another procedure, namely the exothermic oxidation of a mixture of XVb and XIVb with chromium trioxide.

^{3}H-BPMC was also prepared for the purpose of dynamic study according to the method of Hilton and O'Brien.[8]

All these compounds synthesized in the present experiment had been, and will be employed as authentic substances.

Experimental[9]

BPMC (I)——The pure material was obtained by recrystallization of crude sample gifted by Mitsubishi Chem. Ind. Co., Ltd. (Tokyo, Japan).

***o-sec*-Butylphenol (II)**——The crude material was purified by silica gel column chromatography with ether–hexane (1: 1, v/v).

***o-sec*-Butylphenylcarbamate (III)**——A mixture of ethylcarbamate (3.5 g), II (5 g) and ethylbenzene (30 ml) was heated at 110° in an oil bath to remove water in the reagents by azeotropic mixture and then cooled to 100°. Subsequently, Al $(OCH(CH_3)_2)_3$ (about 1.0 g) was added under stirring and then the reaction temperature was raised to and maintained at 135° for 8 hr. After cooling, the mixture was poured onto ice-water and extracted with benzene. After evaporation of the solvent, the resulting crystalline residue was subjected to column chromatography on silica gel (80 g), and the column was eluted with 1 liter of benzene, followed by 100 ml of $CHCl_3$. The $CHCl_3$ eluate afforded 680 mg of III as colorless needles, mp 87—88° (ether–hexane). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 3450 (NH_2), 1745 (CO). NMR ($CDCl_3$) δ (J=Hz): 0.82 (3H, t, J=6.9, $CH_2C\underline{H}_3$), 1.20 (3H, d, J=6.8, $CHC\underline{H}_3$), 1.58 (2H, qt, J=7.2, $C\underline{H}_2CH_3$), 2.86 (1H, sx, J=7.2, $C_6H_5C\underline{H}$), 5.30 (2H, br signal, NH_2), 6.8—7.3 (4H, m, aromatic H). Mass Spectrum *m/e*: 193.1090 (M^+) (Calcd. for $C_{11}H_{15}O_2N$, 193.1089), 150, 121, 107, 103, 91, 77. *Anal.* Calcd. for $C_{11}H_{15}O_2N$: C, 68.37; H, 7.82; N, 7.22. Found: C, 68.35; H, 7.51; N, 7.22.

***o*-(1-Hydroxy-1-methylpropyl)phenol (V)**——*o*-Hydroxyacetophenone (5.0 g) was allowed to react with a Grignard solution prepared by EtI (11.4 g) and Mg. The usual work up gave a crystalline residue, which were purified by passing through a column of silica gel with benzene. Recrystallization from hexane gave 1.2 g of V as colorless prisms, mp 58—59°. NMR ($CDCl_3$) δ (J=Hz): 0.87 (3H, t, J=7.5, $CH_2C\underline{H}_3$), 1.60 (3H, s, C_6H_5-$CC\underline{H}_3$), 1.87 (2H, q, J=7.5, CH_2), 2.54 (1H, br signal, OH), 6.5—7.2 (4H, m, aromatic H), 8.9 (1H, br signal, OH). Mass Spectrum *m/e*: 166.0965 (M^+) (Calcd. for $C_{10}H_{14}O_2$, 166.0994), 138, 133 (base peak), 105, 91.

***o*-(1-Hydroxy-1-methylpropyl)phenyl N-Methylcarbamate (VI)**——To a solution of V (1.0 g) in benzene (30 ml) was added one drop of triethylamine and methylisocyanate (350 mg) and the mixture was allowed to stand for 24 hr at room temperature. After evaporation of the solvent, the obtained syrupy residue was applied to a silica gel column (10 g), which was eluted with 800 ml of benzene, followed by 400 ml of $CHCl_3$. The $CHCl_3$ eluate was evaporated and the residue was recrystallized from ether–hexane to give 560 mg of VI as prisms, mp 73—74°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 3450 (NH), 1730 (CO). NMR ($CDCl_3$) δ (J=Hz): 0.79 (3H, t, J=7.0, $CH_2C\underline{H}_3$), 1.57 (3H, s, $C(OH)C\underline{H}_3$), 1.92 (2H, q, J=7.0, $C\underline{H}_2CH_3$), 2.88 (3H, d, J=5.0, $NHC\underline{H}_3$), 4.97 (1H, br signal, NH), 6.9—7.6 (4H, m, aromatic H). Mass Spectrum *m/e*: 223.1233 (M^+) (Calcd. for $C_{12}H_{17}O_3N$: 223.1208), 205, 194, 166, 148, 137 (base peak), 133, 121, 119, 91. *Anal.* Calcd. for $C_{12}H_{16}O_3N$: C, 64.55; H, 7.68; N, 6.27. Found: C, 64.63; H, 7.69; N, 6.29.

4-Methyl-3,4-dihydrocoumarin (VIII)——This sample was prepared by the procedure reported by Ito, *et al.*[4]

***o*-(3-Hydroxy-1-methylpropyl)phenol[10] (X)**——To a suspension of $LiAlH_4$ (150 mg) in dry ether (15 ml) was added little by little a solution of 1.0 g of 4-methyl-3,4-dihydrocoumarin (VIII) in dry ether (15 ml) stir-

7) G. Wagner, D. Singer, and W. Weuffen, *Pharmazie*, **21**, 161 (1968).
8) B.D. Hilton and R.D. O'Brien, *J. Agr. Food Chem.*, **12**, 236 (1965).
9) All melting points are uncorrected. Infrared (IR) spectra were measured with a spectrometer, Model DS-402 G, Japan Spectroscopic Co., Ltd. NMR spectra were measured with a spectrometer Model 3H-60, Japan Electron Optics Lab., using TMS as an internal standard. Multiplicities of signals are represented as s (singlet), d (doublet), t (triplet), q (quartet), qt (quintet), sx (sextet) and m (multiplet). Mass spectra were taken with a JEOL JMS-OISG-2 mass spectrometer with the ionizing energy at 75 eV, equipped with EI source.
10) R.M. Ladize, *Materialy Nauchn. Konf. Inst. Khim. Akad. Nauk Azerb., Arm. Gruz. SSR. Akad. Nauk Arm. SSR, Inst. Organ. Khim., Erevan*, **1957**, 252 [*C.A.*, **59**, 1544*e* (1965)].

ring at room temperature, and the solution was stirred for more than 1 hr. After decomposition of excess $LiAlH_4$ with H_2O, the reaction mixture was acidified with dil. H_2SO_4 and extracted with ether. The combined ether extract was further extracted with 5% NaOH and the alkaline layer was acidified with dil. H_2SO_4, and extracted with ether. The ether extract was dried, and concentrated. The obtained crystalline mass was recrystallized from benzene–hexane to afford 960 mg of X as colorless needles, mp 67—68°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3610, 3330 (OH). NMR ($CDCl_3$) δ (J=Hz): 1.28 (3H, d, J=7.7, $CHC\underline{H}_3$), 1.3—2.3 (2H, m, $C\underline{H}_2CH_2OH$), 2.9—3.8 (3H, m, overlapping of $CHCH_2C\underline{H}_2OH$ and $C_6H_5C\underline{H}$), 5.04 (2H, s, overlapping of phenolic and alcoholic OH), 6.5—7.2 (4H, m, aromatic H). Mass Spectrum m/e: 166.0986 (M^+) (Calcd. for $C_{10}H_{14}O_2$, 166.0985), 149, 133, 121 (base peak). *Anal.* Calcd. for $C_{10}H_{14}O_2$: C, 72.26; H, 8.49. Found: C, 72.20; H, 8.40.

***o*-(3-Hydroxy-1-methylpropyl)phenyl N-Methylcarbamate (XII)**——To a solution of X (100 mg) in benzene (5 ml) was added one drop of triethylamine and methylisocyanate (35 mg) and the reaction mixture was allowed to stand for 12 hr at room temperature and then heated under reflux for 3 hr. After evaporation of the solvent the residual syrup was submitted to a silica gel plate with MeCN–benzene (1: 9, v/v), on which two dark spots (*Rf's* 0.21 and 0.09) were observed under UV light (254 nm). The spot (*Rf* 0.21) was, furthermore, separated into two spots (*Rf's* 0.11 and 0.08) by TLC on silica gel using ether–hexane (1: 1, v/v). From the spot (*Rf* 0.11), 25 mg of o-(1-methyl-3-N-methylcarbamoyloxypropyl) phenol (XI) was yielded as prisms, mp 81—82°. IR ν_{max}^{KBr} cm^{-1}: 3300 (NH), 3205 (OH), 1700 (CO). NMR ($CDCl_3$) δ (J=Hz): 1.25 (3H, d, J=6.9, $CHC\underline{H}_3$), 1.92 (2H, q, J=6.9, $C\underline{H}_2CH_2O$), 2.80 (3H, d, J=5.1, $NHC\underline{H}_3$), 3.20 (1H, qt, J=6.9, $C_6H_5C\underline{H}$), 4.04, (2H, t, J=6.8, $CH_2C\underline{H}_2O$), 4.71 (1H, br signal, NH), 6.23 (1H, br signal, OH), 6.65—7.29 (4H, m, aromatic H). Mass Spectrum m/e: 223.1168 (M^+) (Calcd. for $C_{12}H_{17}O_3N$, 223.1208), 148, 133 (base peak), 121, 105, 91. From the spot (*Rf* 0.08), a small amount of X was recovered. The material obtained from the lower spot (*Rf* 0.09) was further purified by TLC on silica gel with MeCN–benzene (1: 9, v/v) to give 60 mg of XII as an oily material. IR $\nu_{max}^{liq.\ film}$ cm^{-1}: 1738 (CO). NMR ($CDCl_3$) δ (J=Hz): 1.19 (3H, d, J=7.0, $CHC\underline{H}_3$), 1.83 (2H, q, J=6.2, $C\underline{H}_2CH_2OH$), 2.40 (1H, br signal, OH), 2.86 (3H, d, J=4.8, $NHC\underline{H}_3$), 3.16 (1H, m, $C_6H_5C\underline{H}$), 3.48 (2H, t, J=5.8, $C\underline{H}_2OH$), 5.35 (1H, br signal, $N\underline{H}CH_3$), 6.9—7.3 (4H, m, aromatic H). Mass Spectrum m/e: 223.1192 (M^+) (Calcd. for $C_{12}H_{17}O_3N$, 223.1208), 194, 166 (M^+-CH_3NCO), 148, 133, 121, 83. *Anal.* Calcd. for $C_{12}H_{17}O_3N$: C, 64.55; H, 7.68; N, 6.27. Found: C, 64.58; H, 7.91; N, 6.25. Acetate: XII was acetylated as usual to give XII-acetate as an oily material. IR $\nu_{max}^{liq.\ film}$ cm^{-1}: 1738 (CO). NMR ($CDCl_3$) δ (J=Hz): 1.24 (3H, d, J=6.8, $C_6H_5CHC\underline{H}_3$), 1.98 (3H, s, $OCOCH_3$), 1.96 (2H, q, J=8.2, $C_6H_5CHC\underline{H}_2$), 3.08 (1H, sx, J=7.2, $C_6H_5C\underline{H}$), 4.02 (2H, t, J=6.8, CH_2O), 5.12 (1H, br signal, NH).

***o*-(2-Carboxy-1-methylethyl)phenyl N-Methylcarbamate (XIIIa)**——By the usual procedures, XII (25 mg) was oxidized in AcOH with CrO_3 to give a syrupy residue, which was recrystallized from ether–hexane to yield 17 mg of XIIIa, mp 85—86°. *Anal.* Calcd. for $C_{12}H_{15}O_4N$: C, 60.75; H, 6.37; N, 5.90. Found: C, 60.94; H, 6.49; N, 5.68. Methyl ester (XIIIb): XIIIa was esterified as usual with MeOH–H_2SO_4 to give a methyl ester as an oil, which was completely identical with the compound (XIIIb) (described below).

***o*-(2-Carboxy-1-methylethyl)phenol (IXa)**——VIII (980 mg) was conventionally hydrolyzed with NaOH–EtOH to give a crystalline mass, which was recrystallized from benzene-hexane to yield 850 mg of IXa as prisms, mp 109—111°. Without further purification, this sample was applied to following reaction.

***o*-(2-Methoxycarbonyl-1-methylethyl)phenyl N-Methylcarbamate (XIIIb)**——IXa (800 mg) was esterified with MeOH–H_2SO_4 as usual to give a crude phenol ester (IXb). Without any purification, a mixture of this phenol ester (104 mg), methylisocyanate (80 mg), triethylamine (2 drops) and benzene (30 ml) was refluxed for 3 hr and then the solvent was distilled off. The residue was submitted to preparative TLC on silica gel with hexane–ether (1: 1, v/v) to give 60 mg of XIIIb as needles, mp 44—45°. IR ν_{max}^{KBr} cm^{-1}: 3330 (NH), 1730 (CO). NMR ($CDCl_3$) δ (J=Hz): 1.26 (3H, d, J=7.3, $CHC\underline{H}_3$), 2.4—2.7 (2H, m, CH_2COO), 2.88 (3H, d, J=5.0, $NHC\underline{H}_3$), 3.52 (1H, sx, J=6.8, $C_6H_5C\underline{H}$), 3.63 (3H, s, $OCOCH_3$), 5.20 (1H, br signal, $N\underline{H}CH_3$), 7.0—7.4 (4H, m, aromatic H). Mass Spectrum m/e: 251.1110 (M^+) (Calcd. for $C_{13}H_{17}O_4$, 251.1109), 194 (M^+-CH_3NCO), 162 (base peak), 147, 121. *Anal.* Calcd. for $C_{13}H_{17}O_4N$: C, 62.14; H, 6.82; N, 5.57. Found: C, 62.08; H, 6.74; N, 5.63.

***Z*-and *E*-*o*-(1-Methyl-1-propenyl)phenol (XIVa and XVa)**——A suspension of V (20 g) in 4 N H_2SO_4 (100 ml) was refluxed for 1 hr and extracted with ether. The organic layer was washed with H_2O, dried, evaporated and distilled to give a mixture (14 g) of XIVa and XVa as an oil, bp 98—104°/15 mmHg. Without further purification, this sample was applied to following reaction.

***Z*-and *E*-*o*-(1-Methyl-1-propenyl)phenyl N-Methylcarbamate (XVb and XIVb)**——A mixture (100 mg) of XVa and XIVa was carbamoylated as usual to give syrupy residues, which were developed on a silica gel plate with hexane–ether (1: 1, v/v) to separate XVb and XIVb. The upper zone was cut to give 40 mg of *Z*-*o*-(1-methylpropenyl)phenyl N-methylcarbamate (XVb) as needles (hexane), 92—95°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (NH) and 1740 (CO). NMR ($CDCl_3$) δ (J=Hz): 1.41 (3H, dq, J_1=1.5, J_2=7.5, =CH–$C\underline{H}_3$), 1.91 (3H, m, $C\underline{H}_3$–$C{=}C\langle^{H}_{CH_3}$) 2.83 (3H, d, J=4.2, $NHC\underline{H}_3$), 4.94 (1H, br signal, NH), 5.55 (1H, qq, J_1=1.5, J_2=7.2, =C–H), 7.0—7.3 (4H, aromatic H). Mass Spectrum m/e: 205.1072 (M^+) (Calcd. for $C_{12}H_{15}O_2N$, 205.1103), 148 (M^+-57, base peak), 133, 131, 105, 91, 77. *Anal.* Calcd. for $C_{12}H_{15}O_2N$: C, 70.22; H, 7.37; N, 6.82. Found: C, 70.41; H, 7.53; N, 6.71. The lower zone was cut to give 25 mg of XIVb as an oily material. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (NH), 1735 (CO). NMR ($CDCl_3$) δ (J=Hz): 1.73 (3H, dq, J_1=1.5, J_2=7.2, CH_3–$C{=}C\langle^{C\underline{H}_3}_{H}$),

1.92 (3H, m, $\underline{CH_3}/C=C<^{CH_3}_{H}$), 2.84 (3H, d, J=5.2, NHC$\underline{H_3}$), 4.90 (1H, br signal, NH), 5.51 (1H, dq, J_1=1.5, J_2=7.2, =C–H), 7.0—7.3 (4H, m, aromatic H). Mass Spectrum *m/e*: 205.1124 (M^+) (Calcd. for $C_{12}H_{15}O_2N$, 205.1103), 148 (M^+-57, base peak), 133, 103, 91. *Anal.* Calcd. for $C_{12}H_{15}O_2N$: C, 70.22; H, 7.37; N, 6.82. Found: C, 70.47; H, 7.63; N, 6.72.

***Z*-*o*-(1,2-Epoxy-1-methylpropyl)phenyl N-Methylcarbamate (XVII)**——To a solution of 500mg of the carbamate (XVb) in $CHCl_3$ (30 ml) was added dropwise perbenzoic acid (490 mg) in $CHCl_3$ (5 ml). The reaction mixture was allowed to stand at room temperature overnight, and then washed with 5% NaOH and H_2O, respectively. Evaporation of the solvent left an oily material which was purified on a silica gel plate (*Rf* 0.15) with ether–hexane (1: 1, v/v) to give 470 mg of the compound (XVII) as a very unstable oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (NH), 1743 (CO). NMR ($CDCl_3$) δ (J=Hz): 0.97 (3H, d, J=5.8, CHC$\underline{H_3}$), 1.54 (3H, s, $C_6H_5CC\underline{H_3}$), 2.97 (3H, d, J=5.2, NHC$\underline{H_3}$), 3.19 (1H, q, J=5.8, C$\underline{H}$CH_3), 5.12 (1H, br signal, NHC$\underline{H_3}$), 7.0—7.6 (4H, m, aromatic H). Mass Spectrum *m/e*: 221.1082 (M^+) (Calcd. for $C_{12}H_{15}O_3N$, 221.1052), 177, 164 (M^+-57), 163 (base peak), 147, 121, 120, 119, 105, 91.

***E*-*o*-(1,2-Epoxy-1-methylpropyl)phenyl N-Methylcarbamate (XVI)**——By the procedure described for the epoxide (XVII), XIVb (500 mg) was treated with perbenzoic acid (490 mg). Purification by TLC left 450 mg of the compound (XVI) as an oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (NH), 1743 (CO). NMR ($CDCl_3$) δ (J=Hz): 1.38 (3H, d, J=5.8, CHC$\underline{H_3}$), 1.52 (3H, s, CH_3–C(O)–C), 2.88 (3H, d, J=4.9, NHC$\underline{H_3}$), 3.01 (1H, q, J=5.8, C$\underline{H}$-CH_3), 5.13 (1H, br signal, NH), 6.8—7.6 (4H, aromatic H). Mass Spectrum *m/e*: 221.1065 (M^+) (Calcd. for $C_{12}H_{15}O_3N$, 221.1052), 177, 164, 163, 147, 121, 120, 119, 105, 91.

***o*-(2-Hydroxy-1-methylpropyl)phenyl N-Methylcarbamate (XVIIIa and XXa)**——i) Catalytic reduction of XVII: A solution of XVII (120 mg) in AcOH was hydrogenated over PtO_2. By the usual work up, the obtained residual oil was submitted to TLC on silica gel with ether–hexane (3: 1, v/v) to separate an alkylcarbamate (XXc, 25 mg) and the desired phenolcarbamate (48 mg). The former was obtained as prisms, mp 105—112° (from ether–hexane). IR ν_{max}^{KBr} cm^{-1}: 3340 (NH, OH), 1650 (CO). NMR ($CDCl_3$) δ (J=Hz): 1.10 (3H, d, J=6.5, CH(OCONHCH_3)C$\underline{H_3}$), 1.28 (3H, d, J=7.5, C_6H_5C(H)C$\underline{H_3}$), 2.76 (3H, d, J=5.1, NHC$\underline{H_3}$), 3.20 (1H, qt, J=7.5, C_6H_5C$\underline{H}$), 4.68 (1H, br signal, NH), 5.10 (1H, qt, J=6.5, CHOCO), 6.7—7.2 (4H, m, aromatic H). Mass Spectrum *m/e*: 223.1145 (M^+) (Calcd. for $C_{12}H_{17}O_3N$, 223.1208), 148 (base peak), 133, 121, 107, 91, 77. The latter, a mixture of *erythro*- and *threo-o*-(2-hydroxy-1-methylpropyl)phenyl N-methylcarbamate (XXa, *Rf* 0.12) was obtained as an oily material. *Anal.* Calcd. for $C_{12}H_{17}O_3N$: C, 64.55; H, 7.68; N, 6.27. Found: C, 64.44; H, 7.86; N, 6.26. Acetate (XXb): XXa was converted to its acetate conventionally to give a mixture of *erythro*- and *threo-o*-(2-acetoxy-1-methylpropyl) phenyl N-methylcarbamate (XXb) as an oily material. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (NH), 1745 (CO). NMR ($CDCl_3$) δ (J=Hz) 1.05 (3H, 34%, d, J=6.3, CH(OCOCH_3)C$\underline{H_3}$), 1.09 (3H, 66%, d, J=5.6, CH(OCOCH_3)C$\underline{H_3}$), 1.23 (3H, d, J=6.3, C_6H_5C(H)C$\underline{H_3}$), 1.95 (3H, 66%, s, OCOCH_3), 2.04 (3H, 34%, s, OCOCH_3), 2.88 (3H, d, J=4.5, NHC$\underline{H_3}$), 3.30 (1H, qt, J=5.6, C_6H_5C$\underline{H}$), 5.15 (1H, qt, J=5.3, C$\underline{H}$OCOCH_3). 5.40 (1H, br signal, NH), 7.1—7.3 (4H, m, aromatic H). Mass Spectrum *m/e*: 265.1296 (M^+) (Calcd. for $C_{14}H_{19}O_4N$, 265.1314), 208 (M^+-57), 148, 133 (base peak), 121, 107, 105, 103, 91, 78. ii) Catalytic reduction of XVI: A mixture of *erythro*- and *threo-o*-(2-hydroxy-1-methylpropyl) phenyl N-methylcarbamate (XVIIIa) was yielded from XVI by the procedure described for XVII. *Anal.* Calcd. for $C_{12}H_{17}ON$: C, 64.55; H, 7.68; N, 6.27. Found: C, 64.40; H, 7.92; N, 6.21. Acetate (XVIIIb): XVIIIa was converted to its acetate to give a mixture of *erythro*- and *threo-o*-(2-acetoxy-1-methylpropyl) phenyl N-methylcarbmate (XVIIIb) IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (NH), 1745 (CO). NMR ($CDCl_3$) δ (J=Hz): 1.05 (3H, 43%, d, J=6.3, CH(OCOCH_3)C$\underline{H_3}$), 1.09 (3H, 57%, d, J=5.6, CH(OCOCH_3)C$\underline{H_3}$), 1.22 (3H, d, J=6.3, C_6H_5C(H)C$\underline{H_3}$), 1.95 (3H, 57%, s, OCOCH_3), 2.04 (3H, 43%, s, OCOCH_3), 2.89 (3H, d, J=4.5, NHC$\underline{H_3}$), 3.29 (1H, qt, J=5.6, PhCH), 5.17 (1H, qt, J=5.3, C$\underline{H}$OCOCH_3), 5.18 (1H, br signal, NH), 7.1—7.3 (4H, m, aromatic H). Mass Spectrum *m/e*: 265.1333 (M^+) (Calcd. for $C_{13}H_{19}O_4N$, 265.1314), 208 (M^+-57), 148, 133 (base peak), 107, 91. iii) Catalytic reduction with Raney Ni: Catalytic reduction of an equimolar mixture(100 mg) of XVII and XVI with Raney Ni, W-2 (1 ml), in EtOH gave 65 mg of a mixture of *erythro*- and *threo-o*-(2-hydroxy-1-methylpropyl) phenyl N-methylcarbamate.

***o*-(2-Hydroxy-1-methylpropyl)phenol (XXIa and XIXa)**——i) $LiAlH_4$ reduction of XVII: With $LiAlH_4$ XVII (110 mg) was conventionally converted to a phenol alcohol which was purified on a silica gel plate to give 95 mg of a mixture of *erythro*- and *threo-o*-(2-hydroxy-1-methylpropyl) phenol as an oily material (XXIa). *Anal.* Calcd. for $C_{10}H_{14}O_2$: C, 72.26; H, 8.49. Found: C, 72.04; H, 8.23. Diacetate (XXIb): XXIa was acetylated as usual to give a mixture of *erythro*- and *threo-o*-(2-acetoxy-1-methylpropyl)phenyl acetate (XXIb). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1765 (CO, aromatic), 1733 (CO, aliphatic). NMR ($CDCl_3$) δ (J=Hz): 1.05 (3H, d, J=6.3, CH(OCOCH_3)C$\underline{H_3}$), 1.23 (3H, d, J=7.4, $C_6H_5CC\underline{H_3}$), 1.92 (3H, 17%, s, CHOCOC$\underline{H_3}$), 2.07 (3H, 83%, s, CHOCOCH_3), 2.34 (3H, 83%, s, C_6H_5OCOC$\underline{H_3}$), 2.36 (3H, 17%, s, C_6H_5OCOC$\underline{H_3}$), 3.09 (1H, qt, J=7.9, C_6H_5CH), 5.10 (1H, qt, J=6.8, C$\underline{H}$OCOCH_3), 6.9—7.4 (4H, m, aromatic H). Mass Spectrum *m/e*: 250.1198 (M^+) (Calcd. for $C_{14}H_{18}O_4$, 250.1205), 208, 191, 190, 164, 163, 149, 148, 147, 133, 122, 121. *Anal.* Calcd. for $C_{14}H_{18}O_4$: C, 67.18; H, 7.25. Found: C, 67.64; H, 7.08. ii) $LiAlH_4$ reduction of XVI: XVI (134 mg) was reduced with $LiAlH_4$ to give 120 mg of a mixture of *erythro*- and *threo-o*-(2-hydroxy-1-methylpropyl)phenol (XIXa), which was acetylated as usual to yield 128 mg of a mixture of *erythro*- and *threo-o*-(2-acetoxy-1-methylpropyl) phenyl acetate

(XIXb) as an oily material. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1765 (CO), 1733 (CO). NMR ($CDCl_3$) δ (J=Hz): 1.04 (3H, 57%, d, J=5.0, $CH(OCOCH_3)C\underline{H}_3$), 1.05 (3H 43%, d, J=6.3, $CH(OCOCH_3)C\underline{H}_3$), 1.24(3H, 57%, d, J=6.0, $PhCHC\underline{H}_3$), 1.23 (3H 43%, d, J=7.4, $C_6H_5CHC\underline{H}_3$), 1.92 (3H, 57%, s, $OCOC\underline{H}_3$), 2.07 (3H, 43%, s, $OCOCH_3$), 2.34 (3H, 43%, s, $OCOCH_3$), 2.36 (3H, 57%, s, $OCOCH_3$), 3.13(1H, sx, J=7.9, $PhC\underline{H}$), 5.13 (1H, qt, J=6.8, $C\underline{H}OCOCH_3$), 6.9—7.4(4H, m, aromatic H). Mass Spectrum *m/e*: 250.1183 (M^+) (Calcd. for $C_{14}H_{18}O_4$, 250.1205), 208, 191, 190, 164, 163, 149, 148, 147, 133, 122, 121. *Anal.* Calcd. for $C_{14}H_{18}O_4$: C, 67.18; H, 7.25. Found: C, 67.24; H, 7.19.

***o*-(1-Acetoxymethylpropyl)phenyl N-Methylcarbamate (XXIVb)**——A mixture of V (10 g), methylisocyanate (4 ml), three drops of triethylamine and dry benzene (30 ml) was refluxed for 5 hr. After cooling the solvent was distilled off and the residue was chromatographed on silica gel (50 g). First benzene elute was collected and evaporated to give 7.5 g of a crystalline mass which consisted of a mixture (ca. 1: 1) of *E-o*-(1-methyl-1-propenyl) phenyl N-methylcarbamate (XIVb) and *o*-(1-methylenepropyl) phenyl N-methylcarbamate (XXII). NMR ($CDCl_3$) δ (J=Hz): XIVb; 1.73 (3H, dq, J_1=1.0, J_2=7.2, $=C(H)C\underline{H}_3$), 1.94 (3H, m, $C\underline{H}_3(C_6H_5)C=$), 2.84 (3H, d, J=5.1, $NHC\underline{H}_3$), 4.9 (1H, br signal, NH), 5.51(1H, dq, J_1=1.0, J_2=7.2, $=C(CH_3)\underline{H}$), 7.0—7.3(4H, m, aromatic H). XXII; 1.02 (3H, t, J=7.7, $CH_2C\underline{H}_3$), 2.38 (2H, q, J=7.8, $C\underline{H}_2CH_3$), 2.84 (3H, d, J=5.1, $NHC\underline{H}_3$) 5.0 (1H, br signal, NH), 5.07 (2H, dm, J=9.0, $=CH_2$), 7.0—7.5 (4H, m, aromatic H). These mixture (XIVb and XXII) (300 mg) was epoxidated, reduced, and acetylated by the procedure described for XVIIIb. By repeated TLC of the residue on a silica gel plate with ether–hexane (1: 1, v/v), 32 mg of XXIVb and 42 mg of XVIIIb were obtained. IR (XXIVb) $\nu_{max}^{liq.\ film}$ cm^{-1}: 3320 (NH), 1738—1710 (OCO, NHCO). NMR ($CDCl_3$) δ (J=Hz): 0.83 (3H, t, J=6.8, $CH_2C\underline{H}_3$), 1.70 (2H, qt, J=6.2, $C\underline{H}_2CH_3$), 1.98 (3H, s, $OCOCH_3$), 2.88 (3H, d, J=5.1, $NHC\underline{H}_3$), 3.19 (1H, qt, J=7.2, $C_6H_5C\underline{H}$), 4.22 (2H, d, J=6.9, $C\underline{H}_2OAc$), 5.18 (1H, br signal, NH), 7.05—7.35 (4H, m, aromatic H). Mass Spectrum *m/e*: 265.1359 (M^+) (Calcd. for $C_{14}H_{19}O_4N$, 265.1314, 208 (M^+-57), 190, 166, 148 (base peak), 135, 133, 131, 119, 107, 91, 77. *Anal.* Calcd. for $C_{14}H_{19}O_4N$: C, 63.38; H, 7.22; N, 5.28. Found: C, 63.06; H, 7.33; N, 5.10.

***o*-(1-Carboxypropyl)phenyl N-Methylcarbamate (XXVa) and *o*-(1-methylacetonyl)phenyl N-Methylcarbamate (XXVI)**——A mixture (25 g) of XVIIIa and XXIVa in AcOH was oxidized, as usual, by CrO_3 to give 6 mg of XXVa from acid fraction and 10 mg of XXVI from neutral fraction as an oily material, respectively. XXVa: IR $\nu_{max}^{liq.\ film}$ cm^{-1}; 3340 (NH), 1723 (CONH, COOH). NMR ($CDCl_3$) δ (J=Hz); 0.87 (3H, t, J=6.9, $CH_2C\underline{H}_3$), 1.5—2.4 (2H, m, CH_2), 2.85 (3H, d, J=5.0, $NHC\underline{H}_3$), 3.62 (1H, t, J=7.5, C_6H_5CH), 5.32 (1H, br signal, NH), 6.9—7.5 (4H, m, aromatic H), 7.58 (1H, br signal, COOH). Mass Spectrum *m/e*; 237.0987 (M^+) (Calcd. for $C_{12}H_{15}O_4N$, 237.1001), 180 (M^+-57), 162 (base peak), 134, 119, 107, 91. XXVI: IR $\nu_{max}^{liq.\ film}$ cm^{-1}; 3340 (NH), 1710 (CO). NMR ($CDCl_3$) δ (J=Hz); 1.35 (3H, d, J=7.2, $C\underline{H}_3CH$), 2.02 (3H, s, CH_3CO), 2.88 (3H, d, J=4.8, $NHC\underline{H}_3$), 3.86 (1H, q, J=7.2, $CH_3C\underline{H}$), 5.30 (1H, br signal, NH), 7.1—7.4 (4H, m, aromatic H). Mass Spectrum *m/e*: 179, 164.0759 (M^+-57) (Calcd. for $C_{10}H_{12}O_2$, 164.0837), 121 (base peak), 107, 103, 93, 91, 77.

***o*-(1-Methoxycarbonylpropyl)phenyl N-Methylcarbamate (XXVb)**——Crude XXVa (30 mg) was conventionally converted to its methyl ester (XXVb) by MeOH–H_2SO_4, which was purified on a silica gel plate with ether–hexane (1: 1, v/v) to give XXVb (26 mg) as an oil. IR $\nu_{max}^{liq.\ film}$ cm^{-1}: 3350 (NH), 1725 (CONH, COOMe). Mass Spectrum *m/e*: 251.1158 (M^+) (Calcd. for $C_{13}H_{17}O_4N$, 251.1158), 194.0933 (M^+-57) (Calcd. for $C_{11}H_{14}O_3$, 194.0943), 134, 112, 107, 91, 77. *Anal.* Calcd. for $C_{13}H_{17}O_4N$: C, 62.14; H, 6.82; N, 5.57. Found: C, 62.18; H, 7.02; N, 5.49.

3-Methyl-2H-1,3-benzoxazine-2,4 (3H)-dione (XXVII)——i) This compound was synthesized according to the method described by Wagner, *et al.*[7] ii) To a solution of a mixture (150 mg) of XIVb and XVb in AcOH (15 ml) was added a solution of CrO_3 (15 mg) in a small amount of H_2O. The reaction proceeded exothermically. By the usual procedures XXVII (65 mg) was obtained as colorless needles (from MeOH), mp 148—149°. This sample was concordant, in all respects, with the sample which was synthesized by the above described method.

^{3}H-*o-sec*-Butylphenyl N-Methylcarbamate——*o-sec*-Butylphenol was tritiated according to the method of Hilton and O'Brien[8] and converted as usual to N-methylcarbamate on treatment with methylisocyanate. The resulting ^{3}H-BPMC was purified on a preparative thin-layer chromatographic plate coated with Silica gel HF_{254} (500 mμ thick), developed five times repeatedly with benzene. The spot corresponding to BPMC was cut from the plate and eluted with MeOH–$CHCl_3$ (5: 95, v/v). The extract was evaporated to dryness and dissolved in acetone. Radiochemical purity, as determined by TLC using Silica gel HF_{254} developed with benzene, was higher than 99%. Treatment with 2 N-NaOH for 6 hr at 60°, followed by acidification and extraction with ether gave a 99.1% recovery of the radioactivity, and it was concluded that ^{3}H was introduced into the aromatic ring and not labile in the aqueous solution.[11]

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11) P.G.C. Douch and J.N. Smith, *Biochem. J.*, 125, 395 (1971).