Synthesis of Possible Metabolites of Methylcarbamate Insecticide Chemicals

Hydroxyaryl and Hydroxyalkylphenyl Methylcarbamates

M. Hamdy Balba[1] and John E. Casida

4-Hydroxyaryl methylcarbamates are produced by the monocarbamoylation of the corresponding hydroquinones, which are prepared by persulfate oxidation of the respective phenol, forming a 4-hydroxyaryl sulfate, followed by acid hydrolysis. 2-Isopropoxy-5-hydroxyphenyl methylcarbamate forms in the following reaction sequence which is designed to produce the proper isomeric configuration: 2-benzyloxyphenol; potassium 3-benzyloxy-4-hydroxyphenyl sulfate; 3-benzyloxy-4-isopropoxyphenyl methylcarbamate; 3-hydroxy-4-isopropoxyphenyl methylcarbamate and its bismethylcarbamate; 2-isopropoxy-5-hydroxyphenyl methylcarbamate. Synthesis routes are given for 2-hydroxyphenyl methylcarbamate and 3-(1-hydroxy-1-methylethyl)phenyl methylcarbamate. To obtain the desired isomer of certain products, advantage is taken of protective benzyl groups and/or of steric hindrance in the carbamoylation reaction or in the partial hydrolysis of the bismethylcarbamate. Hydroxylation of the aryl grouping frequently increases the anticholinesterase activity of substituted-aryl methylcarbamates, but usually reduces the acute toxicity to mice, while formation of hydroxyalkylphenyl methylcarbamates apparently does not greatly affect the toxicity or anticholinesterase activity.

Substituted-aryl methylcarbamate insecticide chemicals metabolize to form certain hydroxyaryl and hydroxyalkylphenyl methylcarbamates. Therefore, methods of synthesis of these metabolites are needed to prove their identity and to establish their toxicological significance.

1-Naphthyl methylcarbamate (carbaryl) is metabolized in plants, insects, and mammals, and/or by enzyme systems prepared from insects and mammals to yield, among other products, 4-hydroxyl-1-naphthyl methylcarbamate (4-hydroxycarbaryl) and 5-hydroxy-1-naphthyl methylcarbamate (5-hydroxycarbaryl) (Dorough, 1967; Dorough and Casida, 1964; Kuhr and Casida, 1967; Leeling and Casida, 1966; Oonnithan and Casida, 1966, 1968; Tsukamoto and Casida, 1967b). Reaction of various naphthalenediols and methyl isocyanate under mild conditions yields the corresponding mono- and bismethylcarbamates, but the products often are not easily isolated (Dorough and Casida, 1964). Conditions suitable for the preparation of pure samples of 4-hydroxy and 5-hydroxycarbaryl are reported by Knaak *et al.* (1965). Difficulties arise in preparation of monocarbamates by carbamoylation of unsymmetrical diols because more than one monocarbamate is possible among the products. These difficulties are overcome, in part, by using the benzyl protecting group, because this group is cleaved while the methylcarbamate group is not cleaved under mild catalytic hydrogenolysis conditions (Abdel-Wahab and Casida, 1967; Balba, 1967; Hartung and Simonoff, 1953). Isocyanate reactions are affected by steric hindrance (Arnold *et al.*, 1957) and this steric hindrance, both in the reaction of methyl isocyanate with substituted hydroquinones and in hydrolysis of the bismethylcarbamates formed, is advantageously useful in preparing monomethylcarbamates.

Whenever possible, it is desirable to utilize the identical phenolic intermediates used in synthesis of insecticidal methylcarbamates for the preparation of the hydroquinone intermediates required in the synthesis of the corresponding 4-hydroxyaryl methylcarbamates. Combinations of the aforementioned reactions are useful in preparing 2-isopropoxy-5-hydroxyphenyl methylcarbamate. Conditions for the preparation of 2-hydroxyphenyl methylcarbamate and 3-(1-hydroxy-1-methylethyl)phenyl methylcarbamate are now known.

Division of Entomology, University of California, Berkeley, Calif. 94720

[1] Present address, University of Cairo, Cairo, U.A.R.

ANALYTICAL METHODS

The reaction products were purified by chromatography using a column containing Florisil (50- to 100-mesh, Floridin Co., Hancock, W. Va.) and the procedure described by Krishna *et al.* (1962), or by preparative-scale thin-layer chromatography (TLC) using plates coated with silica gel G (Mallinckrodt Chemical Works, St. Louis, Mo.) at a thickness of 0.5 or 1.0 mm. For preparative-scale TLC, samples of up to 0.2 gram were used and appropriate fluorescent regions, as evidenced by viewing under short wavelength ultraviolet light, were removed by scraping, and the compounds were eluted from the scrapings with methanol. Finally, the purified solid products were recrystallized prior to analysis or testing.

The purity of reaction products was determined by TLC using silica gel F_{254} precoated plates (Brinkman Instruments, Inc., Westbury, N.Y.); all reported R_f values were determined using an ether-hexane (1 to 1) mixture as the developer for hydroquinones and an ether-hexane (4 to 1) mixture for methylcarbamates. Chromogenic reagents used for detection of compounds on the TLC plates were: ninhydrin, after alkaline hydrolysis on the plates, to detect methylcarbamates; and Gibbs' reagent and ferric chloride–potassium ferricyanide, before and after alkaline treatment, to detect the phenolic moiety liberated on alkaline hydrolysis of esters (Krishna *et al.*, 1962).

Elemental analyses, by combustion, were performed by the Microchemical Laboratory, Department of Chemistry, University of California, Berkeley. Infrared spectra were measured, using potassium bromide pellets or chloroform solutions, on a Beckman IR-4 spectrophotometer. The ultraviolet spectra were determined in ethanol solution,

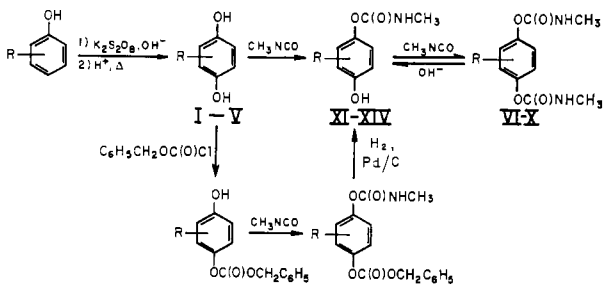
usually at 0.1 m*M* concentration, on a Bausch and Lomb Spectronic 505 spectrophotometer. Refractive indices were determined with an Abbé-type refractometer. All melting points were determined on a micro hot stage and are uncorrected.

The synthesized, purified compounds were compared by cochromatography with labeled metabolites of methylcarbamate-carbonyl-C^{14} insecticide chemicals produced by living plants, or houseflies, or by enzyme preparations from houseflies or rat liver (Kuhr and Casida, 1967; Oonnithan and Casida, 1966, 1968; Shrivastava, 1967; Tsukamoto and Casida, 1967a, b).

Anticholinesterase activity was determined *in situ* on TLC plates coated with silica gel G, using human blood plasma (Oonnithan and Casida, 1966, 1968). The results were expressed as the minimum detectable level (MDL) which shows an inhibitory spot on the assay plate. Male white mice (16 to 19 grams, Berkeley Pacific Laboratories, Berkeley, Calif.) were used for intraperitoneal toxicity studies, in which a solution of the compounds in 50 μl. of dimethyl sulfoxide was injected into each mouse.

METHODS OF SYNTHESIS

4-Hydroxyaryl Methylcarbamates. Reactions used for preparation of hydroquinones, 1,4-bismethylcarbamates, and 4-hydroxyaryl methylcarbamates generally are those given in the following scheme:



The numbered products are identified in Tables I, II, and III.

Hydroquinones (I to V) and 1,4-Bismethylcarbamates (VI to X). Appropriate phenols (30 mmoles) were converted to hydroquinones (I to V, Table I) by Elbs persulfate oxidation according to the method of Baker and Brown (1948) and Aghoramurthy and Seshadri (1952). The ether solution containing the hydroquinone products was evaporated to approximately 25 ml., and the concentrated solution was passed through a Florisil column and eluted quickly with ether because the hydroquinones are not stable on this column. The desired material was found in the first light-yellow band. Contamination from the more slowly moving brown or violet zone must be avoided. Concentration of the eluate and addition of hexane yielded the crystalline hydroquinone. If this procedure, with recrystallization, was insufficient to obtain compounds of reasonable purity, chromatography and recrystallization procedures were repeated. Each hydroquinone gave a single spot on TLC, had the appropriate response to chromogenic reagents, and had the expected infrared spectrum. The respective 1,4-bismethylcarbamates (VI to X), derived on reaction with methyl isocyanate (see below), gave satisfactory analytical values.

The 1,4-bismethylcarbamates were prepared in almost quantitative yields by reaction of the respective hydroquinone (1 mmole) in benzene (50 ml.), containing 1 drop of triethylamine, with methyl isocyanate (10 mmoles) at 20° C. for 18 hours in a closed container. On recrystallization from ethanol, the products gave single spots with anticipated chromogenic responses on TLC, expected infrared spectra, and analytical results approximating theory (Table I).

4-Hydroxyaryl Methylcarbamates (XI to XIV) and 5-Hydroxy-1-naphthyl Methylcarbamate. The respective hydroquinone (10 mmoles) or 1,5-naphthalenediol (10 mmoles) in anhydrous ether (150 ml.), containing 3 drops of triethylamine, was made to react with methyl isocyanate (10 mmoles) at 20° C. for 2 days in a stoppered flask. The reaction mixture was evaporated to dryness, and washed two or three times with ether (50-ml. portions) to leach the desired product from the insoluble residue (bismethylcarbamate). The ether-soluble materials were passed quickly through a Florisil column and eluted with ether, leaving the oxidation products and any residual bismethylcarbamate on the column. Rechromatography of the eluates with hexane, ether-hexane (1 to 1), and ether-hexane (4 to 1) gave the desired product (which was not always pure) in the ether-hexane (4 to 1) eluate. In the case of 2,6-diisopropylhydroquinone and 2,6-dimethylhydroquinone, the two monocarbamates derived from each hydroquinone were separated from each other and from the other reaction products by column chromatography. 1,5-Naphthalenediol gave a single product. The two products from 2-isopropylhydroquinone were not resolved on the column but, subsequently, were separated and isolated by preparative-scale TLC using chromatoplates of 1-mm. thickness and an ether-hexane (4 to 1) mixture. Recrystallization of each product from an ether-hexane mixture gave a pure monomethylcarbamate as evidenced by a single spot on the TLC chromatogram. Elemental analyses (Table I), infrared and ultraviolet spectra, and chromogenic responses were appropriate for each compound.

5-Hydroxy-1-naphthyl methylcarbamate, prepared as above in 24% yield, was identical with that described by Knaak *et al.* (1965) (m.p. 168–70°; lit. 166-67°) (N%, calculated 6.45, found 6.16). It had an R_f of 0.54 on TLC using the ether-hexane (4 to 1) mixture, and the infrared and ultraviolet spectra were appropriate for the assigned structure.

Two isomeric 4-hydroxyaryl methylcarbamates form under these reaction conditions, in ratios varying with each hydroquinone. The desired isomer is the one of lower R_f value with compound XI, and of higher R_f value with compounds XII to XIV. The above-mentioned procedure yields 3-isopropoxy-4-hydroxyphenyl methylcarbamate rather than the desired 2-isopropoxy-4-hydroxyphenyl methylcarbamate as the predominant product on monocarbamoylation of 2-isopropoxyhydroquinone. Therefore, two alternative procedures were used for the preparation of the 2-isopropoxy compound.

Partial alkaline hydrolysis of the bismethylcarbamate was achieved by dropwise addition of aqueous ammonium hydroxide to a solution of isopropoxyphenyl-2,5-bismethylcarbamate (2 mmoles) in methanol (10 ml.) until the alcoholic solution gave a color on pH paper comparable

Table I. Analytical Data for New Aryl Hydroquinones, Aryl-1,4-bismethylcarbamates, and 4-Hydroxyaryl Methylcarbamates

Compound				Elemental Analyses, %		
No.	Name	Yield, %	M.P., °C.	Calcd.	Found	R_f Values for TLC
			ARYL HYDROQUINONES			
I	1,4-Naphthalenediol	15	168–75[a]			0.68
II	2-Isopropoxyhydroquinone	36	112–14			0.38
III	2-Isopropylhydroquinone	33	127.5–30[a]			0.40
IV	2,6-Diisopropylhydroquinone	33	104–7			0.61
V	2,6-Dimethylhydroquinone	35	144–6[a]			0.37
			ARYL-1,4-BISMETHYLCARBAMATES			
VI	Naphthyl-1,4-bismethyl-carbamate	95–100	211–14	C 61.31 H 5.14 N 10.21	61.33 5.18 10.18	0.18
VII	Isopropoxyphenyl-2,5-bis-methylcarbamate	95–100	160–1.5	C 55.31 H 6.43 N 9.92	54.83 6.23 9.88	0.22
VIII	Isopropylphenyl-2,5-bis-methylcarbamate	95–100	151–6	C 58.64 H 6.81 N 10.52	58.56 6.76 10.35	0.28
IX	1,3-Diisopropylphenyl-2,5-bis-methylcarbamate	95–100	195–8	C 62.32 H 7.84 N 9.08	62.16 7.59 9.06	0.51
X	1,3-Dimethylphenyl-2,5-bis-methylcarbamate	95–100	210–12	C 57.13 H 6.39 N 11.10	57.16 6.21 10.86	0.25
			4-HYDROXYARYL METHYLCARBAMATES			
XI	2-Isopropoxy-4-hydroxyphenyl methylcarbamate	26	101–2	C 58.66 H 6.71 N 6.22	58.78 7.05 6.44	0.40
XII	3-Isopropyl-4-hydroxyphenyl methylcarbamate	9	108–9.5	C 63.14 H 7.23 N 6.69	63.50 7.21 6.91	0.55
XIII	3,5-Diisopropyl-4-hydroxyphenyl methylcarbamate	20	146–8	C 66.91 H 8.42 N 5.57	66.68 8.78 5.05	0.79
XIV	3,5-Dimethyl-4-hydroxyphenyl methylcarbamate	22	161–4.5	C 61.53 H 6.71 N 7.17	61.93 6.62 7.41	0.56

[a] The following known compounds have been prepared by other procedures with the following m.p., ° C.: I, 173 (Plimpton, 1880); III, 130-1 (Bayrac, 1895); V, 149-51 (Noelting and Baumann, 1885).

to that of pH 9 to 10, and then heating at approximately 50° C. until the yield of the desired product was at a maximum, as determined by TLC analysis using an ether-hexane (4 to 1) mixture and ninhydrin spray. When 2-isopropoxyhydroquinone was found with the reaction products, the mixture was cooled to 5° C., acidified, and extracted three times with chloroform, and the chloroform extract was dried and evaporated. TLC analysis revealed the presence of four substances in the residual solid: unreacted bismethylcarbamate (R_f = 0.22); two different monomethylcarbamates (R_f = 0.40 and 0.60, each of which gave the chromogenic responses for both carbamoyl and phenolic groups, the one of lower R_f value being in larger amount); and 2-isopropoxyhydroquinone (R_f = 0.90). On monocarbamoylation of 2-isopropoxyhydroquinone, the same two monomethylcarbamates were obtained but, in this case, the product of R_f = 0.60 predominated over the product of R_f = 0.40. Based on the expected steric effect of the isopropoxy group, the product of R_f = 0.40 was assigned the structure of 2-isopropoxy-4-hydroxyphenyl methylcarbamate, and the product of R_f = 0.60 the structure of 3-isopropoxy-4-hydroxyphenyl methylcarbamate. 2-Isopropoxy-4-hydroxyphenyl methylcarbamate (R_f = 0.40), isolated by preparative-scale TLC and recrystallization from ether-hexane mixture (m.p. 101-2° C.), had satisfactory elemental analysis (Table I), infrared and ultraviolet spectra, and chromogenic responses for the assigned structure.

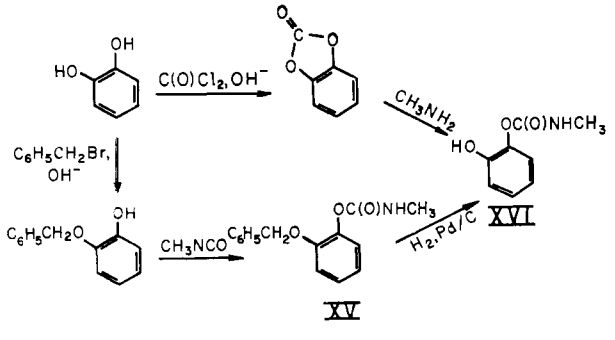
An alternative route for synthesizing 2-isopropoxy-4-hydroxyphenyl methylcarbamate involved the following sequence: 2-isopropoxyhydroquinone → 2-isopropoxy-4-*O*-carbobenzyloxyphenol → 2-isopropoxy-4-*O*-carbobenzyloxyphenyl methylcarbamate which, subsequently, is cleaved by catalytic hydrogenolysis. 2-Isopropoxy-4-*O*-carbobenzyloxyphenol was obtained in 48% yield by refluxing 2-isopropoxyhydroquinone (5 mmoles) with benzylchloroformate (5 to 10 mmoles) in benzene solution (250 ml.) containing pyridine (5 mmoles) for 18 hours followed by cleanup on a Florisil column using benzene for elution. The yellow oily product was made to react with excess

Table II. Analytical Data for 2-Hydroxyphenyl Methylcarbamate, 3-Hydroxyphenyl Methylcarbamate, 2-Isopropoxy-5-hydroxyphenyl Methylcarbamate, 3-(1-Hydroxy-1-methylethyl)phenyl Methylcarbamate, and Certain Methylcarbamate Intermediates Used in Their Preparation

Compound			Elemental Analyses, %		
No.	Name	M.P., °C.	Calcd.	Found	R_f Values for TLC
XV	2-Benzyloxyphenyl methylcarbamate	89.0–90.5	C 70.02 H 5.88 N 5.44	69.89 5.55 5.31	0.70
XVI	2-Hydroxyphenyl methylcarbamate	126.5–7.5	C 57.48 H 5.43 N 8.38	57.36 5.55 8.39	0.32
XVII	3-Hydroxyphenyl methylcarbamate	92–4	C 57.48 H 5.43 N 8.38	57.58 5.89 8.57	0.47
XVIII	3-Benzyloxy-4-isopropoxyphenyl methylcarbamate	105.5–7.5	C 68.55 H 6.71 N 4.44	68.87 6.83 4.35	0.75
XIX	3-Hydroxy-4-isopropoxyphenyl methylcarbamate	125–7	C 58.66 H 6.71 N 6.22	57.89 6.66 6.19	0.52
XX	Isopropoxyphenyl-2,4-bis-methylcarbamate	105–5.5	C 55.31 H 6.43 N 9.92	55.53 6.21 10.13	0.18
XXI	2-Isopropoxy-5-hydroxyphenyl methylcarbamate	99–101	N 6.22	6.58	0.39
XXII	3-(1-Hydroxy-1-methylethyl)phenyl methylcarbamate	92–3	C 63.14 H 7.23 N 6.69	63.37 7.32 6.76	0.39

methyl isocyanate in a closed container to obtain the expected methylcarbamate in 73% yield, after recrystallization from ether-hexane mixture; the crystals (m.p. 91–94° C.) had the expected infrared spectrum. Catalytic hydrogenolysis of 2-isopropoxy-4-*O*-carbobenzyloxyphenyl methylcarbamate (0.75 mmole) in a Parr hydrogenator over 10% palladium-on-charcoal (3 grams) in 2-propanol (50 ml.) at 20° C. and atmospheric pressure, with shaking, gave 2-isopropoxy-4-hydroxyphenyl methylcarbamate (XI) (m.p. 102° C.), in 67% yield. The product was identical (R_f value, infrared spectrum, melting point) with that obtained by partial hydrolysis of the bismethylcarbamate, giving a mixed melting point of 101–2° C.

Other Hydroxyaryl Methylcarbamates. 2-HYDROXYPHENYL METHYLCARBAMATE (XVI). The reaction sequences used in preparing this compound are as follows:

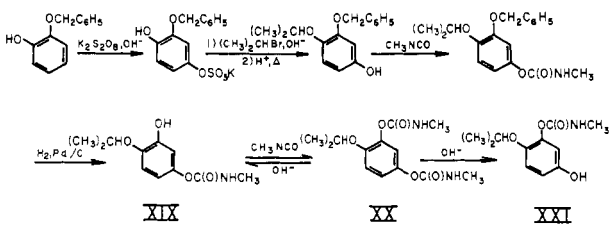


2-Hydroxyphenyl methylcarbamate was prepared by a procedure based on that reported for 2-hydroxyphenyl ethylcarbamate (Petersen, 1949), using methylamine instead of ethylamine. *o*-Phenylene carbonate (3 mmoles) (m.p. 120° C.; lit. 119–20° C.), prepared in 80% yield according to the method of Hanslick *et al.* (1953), was suspended at 5° C. in anhydrous ethanol (1 ml.), and was shaken at 20° C. with methylamine (100 mmoles, 40% aqueous solution) until dissolution of the suspended material took place, and the color changed from colorless to yellow to orange, the final reaction mixture having a pH of 8 to 9. Acidification with hydrochloric acid to pH 1 and extraction three times with chloroform gave the desired material which was recrystallized from an ether-hexane mixture to give a product (92%) having satisfactory elemental analysis (XVI, Table II) and infrared and ultraviolet spectra.

An alternative method for preparation of 2-hydroxyphenyl methylcarbamate followed this sequence: 2-benzyloxyphenol → 2-benzyloxyphenyl methylcarbamate (XV) → 2-hydroxyphenyl methylcarbamate. 2-Benzyloxyphenol was formed by reaction of catechol with benzyl bromide in anhydrous acetone, in the presence of anhydrous potassium carbonate, by the procedure of Klarmann *et al.* (1932). This gave a colorless oil (b.p. 115–20° C. per 0.10 to 0.12 mm.; lit. 157° C. per 6 mm.) in 24% yield which appeared to be a pure compound (TLC), and which had the appropriate infrared spectrum for 2-benzyloxyphenol. Reaction with 10*M* equivalents of methyl isocyanate per mole of 2-benzyloxyphenol, by the procedure previously described, followed by evaporation to dryness and recrystallization from ether-hexane, gave 2-benzyloxyphenyl methylcarbamate (XV) in 78% yield as a pure compound (TLC) with a satisfactory elemental analysis (Table II). Catalytic reduction, on a 0.75-mmole scale for 10 minutes, followed by workup as previously described, gave 2-hydroxyphenyl methylcarbamate (m.p. 125–26° C.) in 88% yield; the product gave a mixed melting point with the compound prepared via aminolysis of *o*-phenylene carbonate of 125–27° C.

3-HYDROXYPHENYL METHYLCARBAMATE (XVII). Reaction of resorcinol and methyl isocyanate by the general procedure described above gave 3-hydroxyphenyl methylcarbamate (XVII).

2-ISOPROPOXY-5-HYDROXYPHENYL METHYLCARBAMATE (XXI). A several-step synthesis, shown in the following scheme, was necessary to assure preparation of authentic 2-isopropoxy-5-hydroxyphenyl methylcarbamate free of isomeric impurities.



XIX XX XXI

Elbs persulfate oxidation, as previously described, of 2-benzyloxyphenol on a 60-mmole scale, gave an aqueous fraction which was adjusted to pH 3, and from which the unreacted 2-benzyloxyphenol was removed by ether extraction. The aqueous solution was adjusted to pH 8 with potassium bicarbonate, concentrated to 70 ml. (using Dow antifoam to minimize foaming), held at 5° C. for 18 hours, and filtered to obtain a crude crystalline product. Recrystallization, first from cold water and then from 90% ethanol, gave light yellow crystals, in 33% yield, which appeared to be a single compound [R_f = 0.70 on TLC using ethyl acetate–2-propanol–water (16 to 6 to 3) mixture], and which gave an infrared spectrum appropriate for potassium 3-benzyloxy-4-hydroxyphenyl sulfate.

Isopropylation of potassium 3-benzyloxy-4-hydroxyphenyl sulfate (12.5 mmoles) was accomplished by dissolving the salt in 50% ethanol (65 ml.), adding 50% aqueous potassium hydroxide (10 ml.) and 2-bromopropane (10 ml.) under reflux, and stirring well for 8 hours to attain thorough mixing. The 2-bromopropane was added intermittently in 1-ml. amounts at intervals needed to maintain a small excess of immiscible oil. Hydrolysis was effected by adding concentrated hydrochloric acid (25 ml.), and heating at 90° C. for 30 minutes. The desired product, a dark yellow oily material, was recovered by cooling, extracting three times with ether, drying the ether extract with anhydrous sodium sulfate, and evaporating to dryness. The product was not further purified, and was found to contain 2-benzyloxyhydroquinone as a minor component (TLC comparison with an authentic specimen) in addition to the major product which appears to be 3-benzyloxy-4-isopropoxyphenol, on the basis of its conversion into the corresponding methylcarbamate by the general procedure previously described. The 3-benzyloxy-4-isopropoxyphenyl methylcarbamate, formed by reaction with methyl isocyanate, was isolated with a Florisil column, eluting with ether-hexane (1 to 2) followed by ether-hexane (3 to 2). Final purification was achieved by recrystallization from ether-hexane. This gave a single product (XVIII) of appropriate response to chromogenic reagents and with the expected infrared spectrum and elemental analysis (Table II) in an over-all yield of 16% based on the amount of potassium 3-benzyloxy-4-hydroxyphenyl sulfate used.

Catalytic reduction of 1.5 mmoles of 3-benzyloxy-4-isopropoxyphenyl methylcarbamate, which required a 35-minute hydrogenolysis under the conditions previously described, yielded 3-hydroxy-4-isopropoxyphenyl methylcarbamate (XIX) in 88% yield, which was found to be pure after recrystallization without benefit of column chromatography. The elemental analysis (Table II), infrared and ultraviolet spectra, and response to chromogenic reagents were as anticipated. Almost quantitative conversion to pure isopropoxyphenyl-2,4-bismethylcarbamate (XX) (single compound on TLC having appropriate chromogenic responses and infrared spectrum) resulted on reaction with a 10-fold excess of methyl isocyanate in the usual manner and recrystallization from ether.

2-Isopropoxy-5-hydroxyphenyl methylcarbamate (XXI) was isolated with difficulty from the hydrolysis products of the bismethylcarbamate which included also 3-hydroxy-4-isopropoxyphenyl methylcarbamate and, probably, 2-isopropoxyresorcinol. A solution of the bismethylcarbamate (0.78 mmole) in methanol (5 ml.) was treated with aqueous ammonium hydroxide solution, as described in the case of the partial hydrolysis of isopropoxyphenyl-2,5-bismethylcarbamate, until the desired product was at a maximum concentration, as determined by TLC. For this determination, aliquots were spotted on the plate, which was then exposed to hydrogen chloride vapors, developed in ether-hexane (4 to 1) mixture, and sprayed with 5% aqueous sodium hydroxide followed by heating at 100° C. for 5 minutes to yield brown spots. Ninhydrin spray was then administered, turning the spots containing carbamates (bismethylcarbamate R_f = 0.18; new compound R_f = 0.39; 3-hydroxy-4-isopropoxyphenyl methylcarbamate R_f = 0.52) to a reddish color. A noncarbamate, probably 2-isopropoxyresorcinol (R_f = 0.65), remained unaffected by ninhydrin. Hydrolysis was allowed to proceed until the compound of R_f = 0.65 was evident, and then the reaction mixture was cooled to 5° C., acidified with hydrochloric acid to below pH 1 (which resulted in a color change from deep yellow to pink), and extracted three times with chloroform. The chloroform extract, after drying with anhydrous sodium sulfate, was evaporated to dryness, and the residual materials were separated on TLC plates of 0.5-mm. thickness. Fluorescent light was used to detect the resolved bands, taking advantage of the fact that the carbamates are poorly fluorescent but increase in fluorescence on standing on the plates, possibly as a result of partial hydrolysis to the substituted resorcinol.

The products were recovered by scraping the appropriate regions of gel, extracting with methanol, filtering, evaporating the methanol, and recrystallizing the products from the ether-hexane mixture. The predominant product was 3-hydroxy-4-isopropoxyphenyl methylcarbamate (XIX), but the desired 2-isopropoxy-5-hydroxyphenyl methylcarbamate (XXI) was isolated, despite the fact that its R_f value is about the same as that of its isomer, in a purity such that only trace amounts of 3-hydroxy-4-isopropoxyphenyl methylcarbamate (XIX) were present as a contaminant. The desired monomethylcarbamate (XXI) was positive to ninhydrin reagent, had the expected R_f value, and gave the appropriate infrared and ultraviolet spectra; also, it was converted by methyl isocyanate to a product with the same R_f value as the bismethylcarbamate. Therefore, it appeared to be an isopropoxy-2,4-disubstituted phenyl com-

pound with one phenolic and one methylcarbamate function. The hydroxyl group was not adjacent to the isopropoxy group, as it did not cochromatograph with authentic compound XIX; so it was considered to be 2-isopropoxy-5-hydroxyphenyl methylcarbamate.

Hydroxyalkylphenyl Methylcarbamate. The reaction sequence was as follows: 3-hydroxyacetophenone → 3-(1-hydroxy-1-methylethyl)phenol → 3-(1-hydroxy-1-methylethyl)phenyl methylcarbamate (XXII). The intermediate, 3-(1-hydroxy-1-methylethyl)phenol, was prepared by reaction of 3-hydroxyacetophenone with excess methylmagnesium iodide. The Grignard reagent was prepared in anhydrous ether (150 ml.) from magnesium (0.2 gram atom) and methyl iodide (0.2 mole) in the usual manner. 3-Hydroxyacetophenone (65 mmoles) in ether (200 ml.) was added dropwise over 1.5 hours to the Grignard reagent at 5° C. with stirring. The reaction was stirred for 18 hours at 20° C., and then refluxed for 2 hours. The reaction mixture was poured into 5*N* hydrochloric acid (50 ml.) containing ice and extracted four times with ether, and the extract was washed with water, 10% aqueous sodium bisulfite, and water. On drying the ether extract over anhydrous sodium sulfate, concentrating, and adding hexane, crystals (m.p. 102–5° C.) were obtained (61% yield), which on recrystallization from ether-hexane mixture gave a pure product identified as 3-(1-hydroxy-1-methylethyl)phenol on the basis of the infrared spectrum, response to chromogenic reagents, and the melting point (104–5° C.) which is similar to the value of 105–6° C. reported for the compound (Auwers, 1916). Auwers (1916) prepared the compound by reaction of 3-hydroxymethylbenzoate with excess methylmagnesium iodide.

The intermediate phenol was converted to 3-(1-hydroxy-1-methylethyl)phenyl methylcarbamate (XXII) by reaction with one molar equivalent of methyl isocyanate in the usual manner. This gave a small amount of ether-insoluble material (possibly the bismethylcarbamate), and one major ether-soluble product which crystallized on addition of hexane to the concentrated ether filtrate, yielding 75% of product (m.p. 88–92° C.). The crystals were washed with hexane and recrystallized from ether-hexane mixture, yielding a product with appropriate elemental analysis (Table II), infrared and ultraviolet spectra, and response to chromogenic reagents.

Catalytic hydrogenolysis of compound (XXII) by the usual procedure gave 3-isopropylphenyl methylcarbamate, based on TLC cochromatography.

ANALYTICAL RESULTS

The analytical results obtained for the synthesized hydroxyaryl and hydroxyalkylphenyl methylcarbamates, and certain intermediates, are given in Tables I and II. Balba (1967) gives details and data pertaining to the infrared and ultraviolet spectra of these compounds.

BIOLOGICAL ACTIVITIES AND COCHROMATOGRAPHY CHARACTERISTICS WITH METABOLITES

Introduction of a hydroxyl group into the ring, without other modification on the molecule, universally results in enhanced anticholinesterase activity but in reduced toxicity to mice, with the exception of 3,5-diisopropyl-4-hydroxyphenyl methylcarbamate, which is more toxic than 3,5-diisopropylphenyl methylcarbamate (Table III). Substitution of a hydroxyl group for the dimethylamino group in 4-dimethylamino-3,5-xylyl methylcarbamate, for the isopropoxy group in 2-isopropoxyphenyl methylcarbamate, and for the isopropyl group in 3-isopropylphenyl methylcarbamate, results in a great reduction in both anticholinesterase activity and toxicity. The biological activity of 3-isopropylphenyl methylcarbamate does not change significantly on introduction of a hydroxyl group into the tertiary position in the alkyl substituent.

Certain hydroxyaryl methylcarbamates are of higher anticholinesterase activity, but of lower toxicity than their analogs lacking the hydroxyl grouping. This difference may result, in part, from the susceptibility of the aryl hydroxyl position to conjugation in the animal body. If this is the case, detoxification by conjugation does not occur as readily with 3-(1-hydroxy-1-methylethyl)phenyl methylcarbamate and 3,5-diisopropyl-4-hydroxyphenyl methylcarbamate because their activity is similar to or higher than the analogs without the hydroxyl groupings, possibly because of the effect of steric hindrance on the biochemical conjugation reaction.

Table III. Comparative Biological Activities of Aryl Methylcarbamates, Hydroxyaryl Methylcarbamates, and Hydroxyalkylphenyl Methylcarbamates

Compound (Name or No.)	Biological Activities: AntiChE MDL, μg.	Biological Activities: IP toxicity to mice, LD_{50}, mg./kg.
1-Naphthyl methylcarbamate (carbaryl)	0.2	29
4-Hydroxy-1-naphthyl methylcarbamate	0.4	74
5-Hydroxy-1-naphthyl methylcarbamate[a]	0.1	56
2-Isopropoxyphenyl methylcarbamate (Baygon)	0.5	12
2-Isopropoxy-4-hydroxyphenyl methylcarbamate (XI)	0.15	52
2-Isopropoxy-5-hydroxyphenyl methylcarbamate (XXI)	0.06	>56
2-Hydroxyphenyl methylcarbamate (XVI)	3.0	>167
3-Isopropylphenyl methylcarbamate (Hercules 5727)	0.004	3.1
3-Isopropyl-4-hydroxyphenyl methylcarbamate (XII)	0.002	48
3-(1-Hydroxy-1-methylethyl)phenyl methylcarbamate (XXII)	0.004	2.9
3-Hydroxyphenyl methylcarbamate (XVII)	2.0	>167
3,5-Diisopropylphenyl methylcarbamate (Hooker HRS-1422)	0.03	17
3,5-Diisopropyl-4-hydroxyphenyl methylcarbamate (XIII)	0.001	11
4-Dimethylamino-3,5-xylyl methylcarbamate (Zectran)	0.02	7.8
4-Hydroxy-3,5-xylyl methylcarbamate (XIV)	0.08	40

[a] Provided by J. B. Knaak, Carnegie-Mellon University, Pittsburgh, Pa.

Among the new compounds synthesized, two cochromatograph with certain metabolites of methylcarbamate insecticide chemicals that are formed in bean plants, the rat liver microsomal oxidase system, houseflies, and/or the microsomal oxidase system derived from houseflies: 2-hydroxyphenyl methylcarbamate from Baygon; 3-(1-hydroxy-1-methylethyl)phenyl methylcarbamate from Hercules 5727 (Kuhr and Casida, 1967; Oonnithan and Casida, 1966, 1968; Shrivastava, 1967; Tsukamoto and Casida, 1967a,b). The site of ring hydroxylation for Baygon is stated to be the 4-position in plants and the 5-position in animal systems. This is not necessarily the case, because the 4- and 5-hydroxy isomers are resolved by TLC chromatography only in certain solvent systems, an authentic sample of the 5-hydroxy isomer not being available at the time the studies on plant metabolism were made (Kuhr and Casida, 1967). Confirmatory evidence on the identity of certain metabolites from the liver microsome system is as follows: The ring-hydroxylated Baygon metabolite cochromatographs with isopropoxyphenyl-2,4-bismethylcarbamate following reaction with methyl isocyanate; hydrogenation of the major metabolite of Hercules 5727 gives 3-isopropylphenylmethylcarbamate, further establishing the site of hydroxylation as on the benzylic carbon (Oonnithan and Casida, 1968). The other hydroxyaryl methylcarbamates prepared are not metabolites, in the aforementioned systems, of the methylcarbamate insecticide chemicals considered here.

DISCUSSION

Methylcarbamoylation of only one hydroxyl group in dihydroxy compounds was achieved, in certain cases, by using appropriate conditions and reactant ratios and, in others, by partial hydrolysis of the bismethylcarbamates. Steric hindrance was an important factor in achieving the desired products. There is a variation in the effect of steric hindrance as evidenced by the methylcarbamate or carbonate which predominates in the following examples: (1) monocarbamoylation of 3-(1-hydroxy-1-methylethyl)-phenol with methyl isocyanate at the phenolic hydroxyl; (2) monoesterification of 2-isopropoxyhydroquinone with benzylchloroformate at the 4-position; (3) monocarbamoylation of 2- and/or 2,6-substituted hydroquinones at the 4-position; and (4) partial alkaline hydrolysis of isopropoxyphenyl-2,5-bismethylcarbamate at the 5-position. In general, the product resulting from reaction at the unhindered position was in higher amount than that at the hindered position, as demonstrated by TLC and by the use of appropriate chromogenic reagents. An exception to reaction 4 occurs with isopropoxyphenyl-2,4-bismethylcarbamate where the more hindered carbamoyl group is the one largely hydrolyzed. Both steric and electronic considerations appear to be involved in the relative rates of hydrolysis of the methylcarbamoyl groups in the isopropoxyphenylbismethylcarbamates. In reaction 1, the steric hindrance of the tertiary position apparently overcomes the nucleophilicity difference between the aromatic and aliphatic hydroxyl groups, so that the aromatic carbamoylation is predominant.

The R_f values are also useful in determining the carbamoylated site on alkyl- or alkoxyhydroquinones or alkoxyresorcinols because, in each case, the product with the unhindered hydroxyl group is of lower R_f than the hindered product. This effect may be due to a greater hydrogen bonding to the silica gel surface in the case of compounds with unhindered hydroxyl groups and a hindered carbamate group, with the result that development is slower than when the groupings and the hydrogen bonding effects are reversed.

The desired hydroquinones were most conveniently prepared by persulfate oxidation of the corresponding phenols with a free para position (Baker and Brown, 1948; Sethna, 1951). The attack is expected only in the para position, and this was confirmed in the present work where known hydroquinones were prepared (1,4-naphthalenediol, 2-isopropylhydroquinone, and 2,6-dimethylhydroquinone), and in the conversion of 2-isopropoxyphenol and 3-isopropoxyphenol to an identical product, 2-isopropoxyhydroquinone. The successful use of persulfate oxidation in preparing 1,4-naphthalenediol is contradictory to the report of Baker and Brown (1948).

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