[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF SOUTH CAROLINA]

Studies in the Biphenyl Series. VI. Orientation in Bromination of 4-Ethoxybiphenyl and Bromo-4-ethoxybiphenyls[1]

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A study has been made on the directive effects of substituent groups in the biphenyl nucleus toward bromination of 4-ethoxybiphenyl and certain bromo-4-ethoxybiphenyls. Substitution occurs at the 3-, 5- and 4'-positions.

From the results of bromination of 4-hydroxybiphenyl, its esters, methyl ether, and their halo derivatives, it is evident that the 3-, 5- and 4'-positions of the biphenyl nucleus are the ones activated. According to well-known theory, electrophilic attacks of bromine are facilitated by relatively high electron densities in these three positions. The solvent plays an important role in orientation of an entering substituent into these positions as is evident in the cases of acetic acid and carbon tetrachloride, which produce different directive effects in halogenation of 4-hydroxybiphenyl[3,4] and 4-acetoxybiphenyl.[5,6] Furthermore, the nature of the group has an effect on the particular position of entry, since replacement of acetoxy with benzoxy[4] or benzenesulfonoxy[7] can also produce different results.

Monobromination of 4-methoxybiphenyl[8,9] in chloroform has been shown to produce a mixture of isomers, both of which yield the same dibrominated derivative when further brominated in this solvent. The present work extends the investigation of orientation effects to 4-ethoxybiphenyl and certain bromo-4-ethoxybiphenyls. Bromination of 4-ethoxybiphenyl (I) in chloroform yielded a mixture of 3-bromo-4-ethoxybiphenyl (II) and 4-ethoxy-4'-bromobiphenyl (III), but only II was obtained in carbon tetrachloride or acetic acid. This difference is probably due to the difficulty of a tedious separation in the latter instances, rather than the non-existence of III among the reaction products. Proofs of the structures of II and III were given by ethylation of known 3-bromo-4-hydroxybiphenyl[5] and 4-hydroxy-4'-bromobiphenyl.[4] Further bromination of II and III yielded 3,4'-dibromo-4-ethoxybiphenyl (IV). Again, the structure of IV was established by ethylation of the known 3,4'-dibromo-4-hydroxybiphenyl.[3]

Direct dibromination of I produced IV, identical with the product formed by bromination of II and III. Tribromination of I yielded 3,5,4'-tribromo-4-ethoxybiphenyl (V). This ether (V) was also formed on bromination of IV and of 3,5-dibromo-4-ethoxybiphenyl (VI). The structures of V and VI were established through ethylation of the corresponding brominated 4-hydroxybiphenyls.[4,10]

(1) Taken from a thesis of Henry Pollock submitted in partial fulfillment of the requirements of the degree of Master of Science.

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(3) Hazlet and Hensley, THIS JOURNAL, **69**, 708 (1947).

(4) Hazlet, Alliger and Tiede, *ibid.*, **61**, 1447 (1939).

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Experimental Part

4-Ethoxybiphenyl.—The 4-ethoxybiphenyl[11,12] (m.p. 72–73°) used in the orientation experiments was prepared in 81% yield by the action of ethyl sulfate on 4-hydroxybiphenyl in alkaline solution.

Ethylation of Bromo-4-hydroxybiphenyls.—Bromoethoxybiphenyls were prepared also by the ethylation of the corresponding phenols. Procedures for the preparation of these compounds were similar. Therefore, details are given for one only. Pertinent data for this and the other ethers, all of which were recrystallized from ethanol, are shown in tabular form. Five grams of 3-bromo-4-hydroxybiphenyl[5] was suspended in 7 ml. of 10% aqueous sodium hydroxide and heated until a clear solution resulted at the temperature of reflux. A 100% excess (3.75 g.) of ethyl sulfate was added dropwise over a period of 15 minutes. The mixture was refluxed for 2 hours and then poured into 200 ml. of cold water. The white solid which formed was removed by filtration and dried with the production of 4.2 g. of 3-bromo-4-ethoxybiphenyl, melting at 73–74°. This amounted to a 75.5% yield. Recrystallization from ethanol did not change the melting point.

Anal. Calcd. for $C_{14}H_{13}OBr$: Br, 28.63. Found: Br, 28.62; 28.43.

ETHYL ETHERS PREPARED FROM KNOWN BROMO-4-HYDROXYBIPHENYLS

Biphenyl	Bromine, % Calcd.	Found	M.p., °C.	Yield, %
3-Bromo-4-ethoxy-	28.63	28.62, 28.43	73–74	75.5
4-Ethoxy-4'-bromo-	28.63	28.56, 28.47	137–138	84.4
3,4'-Dibromo-4-ethoxy-	44.63	44.45, 44.46	100–100.5	66.5
3,5-Dibromo-4-ethoxy-	44.63	44.53, 44.34	59–61	86.2
3,5,4'-Tribromo-4-ethoxy-	55.24	54.99, 54.48	99.5–101	77.4

Monobromination of 4-Ethoxybiphenyl. A. In Chloroform.—Twenty grams of 4-ethoxybiphenyl was dissolved in 75 ml. of freshly distilled and dried chloroform, to which had been added approximately 0.3 g. of iron powder. A solution of 17.5 g. of bromine in 25 ml. of chloroform was added dropwise, with stirring, during a period of 45 minutes. The evolution of hydrogen bromide ceased shortly after all the bromine had been added. The solution was refluxed for 30 minutes and then cooled. A 5% solution of sodium thiosulfate (75 ml.) was added and stirring was continued until the chloroform solution became pale yellow in color. The aqueous layer was removed and the chloroform solution was washed with 300 ml. of water. The chloroform solution was dried for 24 hours over anhydrous calcium chloride, the chloroform was removed by distillation and the oily residue was dissolved in 150 ml. of hot ethanol. The solid which formed upon cooling was removed by filtration and when dry yielded 19.3 g. of white, irregular, crystals, which melted at 54–63°. Several recrystallizations from both ethanol and petroleum ether gave 2.5 g. of 3-bromo-4-ethoxybiphenyl melting at 73–74°. A mixture of this ether with the product formed by ethylation of 3-bromo-4-hydroxybiphenyl[5] caused no depression in melting point. The material which was insoluble in ethanol could not be separated into pure component compounds by recrystallization.

(11) Musser and Adkins, THIS JOURNAL, **60**, 664 (1938).

(12) Brewster and Putman, *ibid.*, **61**, 3083 (1939).

Another bromination of 20 g. of 4-ethoxybiphenyl yielded 18.1 g. of a solid melting at 51–62°. This was subjected to extraction in a Soxhlet type apparatus, using 25 ml. of a gasoline fraction (b.p. 40–45°). The solvent was refluxed for 2 hours over a hot water-bath and then cooled at the close of the extraction. The residue in the porous cup was recrystallized to produce 4.1 g. (platelets) of 4-ethoxy-4'-bromobiphenyl, with a melting point of 137.5–138.5°, as proven by a mixed melting point with known 4-ethoxy-4'-bromobiphenyl. The crystals which formed from the solvent upon cooling were removed by filtration and upon drying yielded 7.8 g. of solid melting at 112–123°. This could not be separated. The extraction solvent from the original extraction was evaporated and the residue upon crystallization from ethanol gave 2.8 g. of solid (m.p. 69–72°) and this yielded 2.0 g. of 3-bromo-4-ethoxybiphenyl (needles), melting at 73–74°. Both 4-ethoxy-4'-bromobiphenyl and 3-bromo-4-ethoxybiphenyl were identified by melting points of mixtures of these products with the respective ethers formed by ethylation of known 4-hydroxy-4'-bromobiphenyl[7] and 3-bromo-4-hydroxybiphenyl.[5]

B. **In Carbon Tetrachloride.**—Bromine (16.2 g.) dissolved in 30 ml. of carbon tetrachloride was added dropwise over a period of 45 minutes, with stirring, to a solution of 20 g. of 4-ethoxybiphenyl in 100 ml. of carbon tetrachloride, which contained 0.3 g. of suspended iron powder as a catalyst, while the solution was heated under reflux. Heating and stirring were continued for 2 hours after bromine addition was complete. Five grams of powdered decolorizing carbon was added with continued heating and stirring for 30 minutes and the hot solution was then filtered. The resulting clear solution was then cooled and dried over 10 g. of anhydrous calcium chloride for a period of 18 hours. The carbon tetrachloride was removed and the oily residue which remained, after removal of the solvent by distillation, was dissolved in 150 ml. of hot ligroin (b.p. 65–110°). The irregular crystals which formed upon cooling were removed by filtration and when dried amounted to 16 g. (m.p. 51–60°). Five recrystallizations from petroleum ether gave 2.7 g. of 3-bromo-4-ethoxybiphenyl (m.p. 73–74°). A mixture of this and known ether prepared from 3-bromo-4-hydroxybiphenyl[5] did not alter the melting point.

C. **In Glacial Acetic Acid.**—A solution of 18.2 g. of bromine in 25 ml. of glacial acetic acid was added with stirring during a 45-minute period to a refluxing solution of 4-ethoxybiphenyl (20 g.) in 100 ml. of glacial acetic acid, to which had been added 0.3 g. of iron powder. Heating and stirring were continued for two hours after the bromine addition was complete. Intermittent evolution of hydrogen bromide was noticed. Five grams of decolorizing carbon was then added and the mixture was heated and stirred for an additional 30 minutes. The hot solution was filtered, cooled and poured into one liter of cold water. A solid formed after 2 hours. This was removed by filtration and dried to give 18.0 g. of a yellow solid. It was then powdered and dissolved in 150 ml. of hot petroleum ether (b.p. 65–110°). Irregular crystals resulted upon cooling. They were removed by filtration and when dry weighed 12.5 g. (m.p. 54–62°). Four recrystallizations from the same solvent yielded 3.1 g. of 3-bromo-4-ethoxybiphenyl, with a melting point of 72–74°. A mixture of this substance with the ether produced by ethylation of known 3-bromo-4-hydroxybiphenyl[5] did not change the melting point.

Dibromination of 4-Ethoxybiphenyl. A. In Chloroform.—Bromine (34.3 g.) dissolved in 35 ml. of chloroform was added dropwise with stirring to a solution of 20 g. of 4-ethoxybiphenyl in 75 ml. of boiling chloroform, to which had been added 0.3 g. of iron powder. After heating, then washing with sodium thiosulfate solution and subsequently with water, followed by drying over anhydrous calcium chloride, the chloroform was removed and 19.5 g. (54.4% yield) of 3,4'-dibromo-4-ethoxybiphenyl (m.p. 99–100.5°) was obtained by crystallization from ethanol. A melting point determined on a mixture with the ethyl ether formed from known 3,4-dibromo-4-hydroxybiphenyl[5] established the structure.

B. **In Carbon Tetrachloride.**—A solution of 34.3 g. of bromine in 25 ml. of carbon tetrachloride was added dropwise to 20 g. of 4-ethoxybiphenyl in 75 ml. of refluxing carbon tetrachloride which contained 0.3 g. of iron powder in suspension. At the end of the heating period, and after clarification of the solution, the solvent was removed and the product was crystallized from ethanol to yield 18.0 g. (50.2% yield) of 3,4'-dibromo-4-ethoxybiphenyl (m.p. 99–100.5°). A melting point of a mixture of this product with the ether formed by ethylation of known 3,4'-dibromo-4-hydroxybiphenyl[3] proved its identity.

C. **In Glacial Acetic Acid.**—Bromine (34.3 g.) was added to 20 g. of 4-ethoxybiphenyl in glacial acetic acid which contained 0.3 g. of iron powder. After heating, the mixture was poured into cold water and the insoluble solid weighed 26.5 g. when dry. This solid was clarified in carbon tetrachloride with decolorizing carbon, the solvent was removed and the oil was crystallized from ethanol to yield 16 g. (44.6% yield) of 3,4'-dibromo-4-ethoxybiphenyl, proven by a melting point of a mixture of this substance with the ethylation product of known 3,4'-dibromo-4-hydroxybiphenyl.[3]

Tribromination of 4-Ethoxybiphenyl.—A solution of 53.4 g. of bromine in 35 ml. of chloroform was added dropwise over a period of 90 minutes, with stirring, to 20 g. of 4-ethoxybiphenyl dissolved in 75 ml. of refluxing chloroform, to which had been added 0.3 g. of iron powder. Heating and stirring were continued for 2.5 hours, until hydrogen bromide was no longer evolved. The mixture was cooled, 100 ml. of 5% aqueous sodium thiosulfate was added and the mixture was stirred until the chloroform layer became pale yellow in color. The chloroform solution was separated and washed with 300 ml. of water and dried over anhydrous calcium chloride. The chloroform was removed by distillation and the oil which remained was dissolved in 200 ml. of hot ethanol. The crystals, needles, which formed upon cooling were removed by filtration and weighed 13.0 g. when dry. This was a 31.6% yield of 3,5,4'-tribromo-4-ethoxybiphenyl (m.p. 99.5–101°) as shown by melting with ether of known structure through ethylation of 3,5,4'-tribromo-4-hydroxybiphenyl.[10] However, a mixture of this tribromo ether, as prepared in this bromination experiment, with some known 3,4'-dibromo-4-ethoxybiphenyl melted at 84–95°.

Bromination of 3-Bromo-4-ethoxybiphenyl: 3,4'-Dibromo-4-ethoxybiphenyl.—A solution of bromine (3.0 g.) in 10 ml. of chloroform was added dropwise during a period of 15 minutes to a refluxing solution of 5 g. of 3-bromo-4-ethoxybiphenyl in 20 ml. of chloroform, to which had been added 0.3 g. of iron powder. The mixture was cooled after refluxing for an additional 2-hour period, washed with 50-ml. of 5% aqueous sodium thiosulfate and then 150 ml. of water, filtered and dried over anhydrous calcium chloride for 12 hours. The chloroform was removed by distillation and the residual oil was dissolved in 100 ml. of hot ethanol. The crystals (platelets) which deposited on cooling were removed by filtration and when dry amounted to 4.7 g. (73.1% yield) with melting point 99.5–100.5°. This was 3,4'-dibromo-4-ethoxybiphenyl, as evidenced by the melting point of a mixture of this substance with the ethylation product of known 3,4'-dibromo-4-hydroxybiphenyl.[3]

Bromination of 4-Ethoxy-4'-bromobiphenyl: 3,4'-Dibromo-4-ethoxybiphenyl.—Five grams of 4-ethoxy-4'-bromobiphenyl was brominated in chloroform with slightly more than an equal molal quantity of bromine and a small amount of iron catalyst. Following the usual procedures, 2.8 g. (43.5%) of 3,4'-dibromo-4-ethoxybiphenyl (m.p. 99–100.5°) was obtained on crystallization from ethanol. This was proven through an identical melting point of a mixture of this ether with the ether formed by ethylation of 3,4'-dibromo-4-hydroxybiphenyl.[3]

Bromination of 3,4'-Dibromo-4-ethoxybiphenyl: 3,5,4'-Tribromo-4-ethoxybiphenyl.—A solution of 6 g. of 3,4'-dibromo-4-ethoxybiphenyl in chloroform was brominated with 3 g. of bromine, using an iron catalyst in the usual way. After the usual treatment, 3.2 g. (44.5% yield) of 3,5,4'-tribromo-4-ethoxybiphenyl, melting at 99.5–100°, was obtained upon recrystallization from alcohol. A melting point of a mixture of this substance with ether known by ethylation of 3,5,4'-tribromo-4-hydroxybiphenyl[10] caused no depression.

Bromination of 3,5-Dibromo-4-ethoxybiphenyl: 3,5,4'-Tribromo-4-ethoxybiphenyl.—Three grams of 3,5-dibromo-ethoxybiphenyl was dissolved in 15 ml. of hot chloroform and to this was added 1.5 g. of bromine in 10 ml. of chloroform over a period of 15 minutes. Iron powder (0.3 g.) was added as a catalyst. The mixture was refluxed for 2 hours after bromine addition was complete, washed with 25 ml. of 5% aqueous sodium thiosulfate and 100 ml. of water and dried over anhydrous calcium chloride for 12

hours. The chloroform was removed by distillation and the oily residue was dissolved in 35 ml. of hot ethanol. The needles which formed upon cooling were removed by filtration and dried to produce 1.2 g. of 3,5,4'-tribromo-4-ethoxybiphenyl with melting point 100–101°. A mixture of this material with some made by ethylation of 3,5,4'-tribromo-4-hydroxybiphenyl[10] did not change the melting point. The yield was 33.0% of the theoretical.

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The Preparation and Properties of Mammalian Ribonucleic Acids[1]

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A method, which is of general application to mammalian tissues, is described for preparing purified ribonucleic acids. The mammalian ribonucleic acids have been characterized with respect to mononucleotide analytical composition as well as nitrogen and phosphorus content. The extent of liberation of titratable phosphate groups by ribonuclease has been determined. Preliminary experiments with the analytical ultracentrifuge reveal that the nucleic acid preparations exist essentially as single sedimenting boundaries.

A method for the preparation of mammalian ribonucleic acids employing high concentrations of guanidine hydrochloride in the fractionation procedure is reported in this paper. Aside from the facility of preparation the method has the advantage of avoiding conditions of acidity and alkalinity which might degrade tissue ribonucleic acids and, by virtue of the protein denaturant action of guanidine salts, of minimizing the possibility for enzymatic degradation.

Ribonucleic acids prepared from yeast by alkaline extraction are reported to have a molecular weight of about 17,000[2,3] while a highly labile ribonucleic acid prepared from tobacco mosaic virus by heat denaturation had a molecular weight of about 300,000.[4] Relatively high molecular weight ribonucleic acids have been prepared from viruses by Markham, *et al.*,[5] and from bacterial surfaces by Stacey.[6] Pancreas ribonucleic acid has been prepared by Levene and Jorpes,[7] Hammarsten,[8] Kerr and Seraidarian,[9] and Allen and Bacher.[10] However, the particle size or homogeneity of these latter preparations was not assessed.

In view of the apparent dependence of the composition of ribonucleic acids upon the methods of preparation as well as the source[9,10] certain considerations appear necessary for a proper assessment of physical and chemical descriptions of ribonucleic acid preparations: (a) The preparative procedure and source, (b) a statement of the physical-chemical homogeneity of the ribonucleic acid, (c) a description of the degradation of the ribonucleic acid by ribonuclease, and (d) a statement of the mononucleotide composition of the ribonucleic acid. It is with reference to these considerations that mammalian ribonucleic acids prepared by the guanidine salt procedure hereinafter described have been studied.

Experimental and Results

Method of Preparation of Mammalian Tissue Ribonucleic Acid.—The method of isolation of ribonucleic acid from tissue homogenates consisted of (a) the removal of desoxyribonucleic acid as a nucleic acid–protein complex,[11] (b) the precipitation of the ribonucleic acid from a cold 2 *M* guanidine hydrochloride solution in which the large bulk of protein remains soluble, and (c) further purification of the ribonucleic acid by chloroform extraction[12] and alcohol precipitations.

The possibility of the occurrence of nuclease action on ribonucleic acid during the preliminary steps of the preparation can be obviated by immediately homogenizing the tissue in concentrated guanidine hydrochloride. The latter reagent is an effective protein denaturant. These procedures were found to be applicable to a number of mammalian tissues; details of the methods of preparation follow.

Fresh or frozen tissue was cut in small pieces and blended for 6 to 8 minutes with 3 volumes per gram tissue of a 0.15 *M* sodium chloride–0.02 *M* phosphate buffer, *p*H 6.8. A few drops of octyl alcohol were added to reduce foaming. The homogenate was then centrifuged at 3000 g. for 30 minutes. Essentially all the desoxyribonucleic acid was removed in the form of an insoluble nucleic acid–protein complex as described by Mirsky and Pollister.[11] All operations were carried out between 2 and 5°.

To the supernatant solution enough solid guanidine hydrochloride was added, with rapid stirring, to make the solution 2 molar with respect to guanidine hydrochloride. The solution was placed in a 38° bath and allowed to stand at this temperature for 30 minutes, then chilled at 0° for 1 hour. Under these conditions most of the protein of the tissue extract remained soluble, while a gelatinous precipitate formed which contained ribonucleic acid and a small amount of protein. The precipitate was washed twice with a cold solution of 2 *M* guanidine hydrochloride in *p*H 6.8 saline–phosphate buffer. By this process any desoxyribonucleoprotein which remained soluble in the high guanidine concentration was removed by the washing process. To remove the contaminating protein the precipitate was then suspended in 2 *M* guanidine hydrochloride (one volume per gram of original tissue) and extracted with chloroform–octyl alcohol (5:1).[11] The suspension of nucleic acid in guanidine hydrochloride was added to an equal volume of the chloroform:octyl alcohol mixture, warmed to 40°, then shaken mechanically for 30 minutes. The mixture was centrifuged and the upper aqueous layer containing the nucleic acid removed. The extraction of the aqueous solution at 40° was repeated twice with fresh chloroform:octyl alcohol. Extractions in the cold, or in saline or water solutions, resulted in incomplete separation of the nucleic acid from protein. Nucleic acid was precipitated in the cold from the guanidine solution by adjusting the acidity to *p*H 4.2–4.5 with acetic acid and adding two volumes of cold ethanol. The white, flocculent ribonucleic acid precipitate was cen-

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