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HIGH-PRESSURE LIQUID CHROMATOGRAPHY OF SOME SUBSTITUTED BIPHENYLS

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SUMMARY

The high-pressure liquid chromatographic analysis of a series of biphenyls, substituted at positions 2-, 3-, 4-, 4,4'-, and 3,4-, has been carried out in the three common modes: adsorption, normal partition, and reversed-phase partition. Ultraviolet absorption monitoring of the effluent was used as the method of detection. Capacity ratios for the derivatives have been determined in the three modes, where possible, and a discussion of the effects of the various substituents upon the retention times is presented. The utility of the three modes of chromatography in separating some mixtures of these biphenyl derivatives is illustrated.

INTRODUCTION

We have been interested in using the techniques of radioimmunoassay (RIA) to monitor exposure to chemical carcinogens such as the aromatic amines benzidine and 4-aminobiphenyl. This required the synthesis of a wide variety of biphenyl derivatives of the two parent amines for use in the development of the RIA procedures; *i.e.*, haptens for conjugation to carrier proteins for use in the development of antibodies, derivatives suitable for iodination to yield radiolabeled haptens and various compounds referred to as inhibitors for testing the specificity of the antibodies.

We decided to use high-pressure liquid chromatography (HPLC)[1,2] in conjunction with other analytical techniques such as thin-layer chromatography, elemental analysis, mass spectrometry, infrared and ultraviolet spectrophotometry, and nuclear magnetic resonance spectroscopy to establish homogeneity and purity of each compound. In addition, HPLC allowed us to scan rapidly a reaction mixture and monitor it for product formation. We present in this paper a description of the behavior of these biphenyl compounds in three liquid chromatographic modes.

EXPERIMENTAL

Chromatography equipment

A Varian 4200 series liquid chromatograph (Varian Assoc., Palo Alto, Calif.,

U.S.A.) was used throughout these studies. The instrument was equipped with two 250-ml syringe pumps and a solvent programmer for gradient-elution analysis. The UV–visible detector was a Varian Variscan (Model 635) unit. The wavelength could be varied continuously from 210 to 780 nm. The system was equipped with a stop-flow valve so that an individual component could be isolated in the micro-UV cell and its qualitative spectrum recorded. Peak elution patterns were recorded on a Hewlett-Packard (Palo Alto, Calif., U.S.A.) Model 7127A recorder.

Columns

All columns used were obtained from Whatman (Clifton, N.J., U.S.A.) and were uniform in size (4.6 mm I.D. × 25 cm). Normal adsorption chromatography was carried out on a column pre-packed with Partisil 10-PXS, a 10-μm silica. For normal partition chromatography a column pre-packed with Partisil 10-PAC, a 10-μm diameter bonded-phase "cyano-type" (CN) material, was used. Reversed-phase partition chromatography was carried out using a column pre-packed with Partisil 10-ODS, octadecylsilane bonded to 10-μm silica through Si–O–Si linkages.

Solvents and chemicals

Organic solvents used in these studies were "distilled in glass" and purchased from Burdick & Jackson Labs. (Muskegon, Mich., U.S.A.). Each solvent was filtered prior to use through a Millipore "MF-Millipore" 0.22-μm GSWP, or "Fluoropore" 0.45-μm HAWP filter, as applicable (Millipore, Bedford, Mass., U.S.A.). Water was distilled and also Millipore-filtered (System R015). Tetrahydrofuran (THF) or dimethylformamide (DMF) was used for the preparation of samples for injection into the liquid chromatographic column. Both THF and DMF were distilled before use and stored under nitrogen.

Several of the compounds used in this study were obtained from a commercial source: 4-nitrobiphenyl, 4-aminobiphenyl, 3-nitrobiphenyl, 4,4′-dinitrobiphenyl, and 2-aminobiphenyl (Aldrich, Milwaukee, Wis., U.S.A.); benzidine was a gift from the National Center for Toxicological Research (Jefferson, Ark., U.S.A.). In a few cases a sample of the commercial amine was trifluoroacetylated and purified. The amine was regenerated by cleavage of the trifluoroacetyl group with alkali. All of the other compounds were synthesized in our laboratory according to standard chemical procedures; they were purified, in most cases, as analytically pure samples. Details of the syntheses of any of these compounds may be obtained from the authors.

Method and operating conditions

Many of the compounds used in this study are quite insoluble in most solvents. Therefore, we have routinely used THF or DMF to prepare samples for injection. Compounds were prepared as approximately 1 mg/ml solutions in one of the indicated solvents. Samples were stored in the dark at 4°; they were prepared fresh at weekly intervals.

The liquid chromatograph was operated at ambient temperature. The column was equilibrated with the solvent system of choice, and a solution of each compound was injected. Peaks were detected at a wavelength preset on the variable-wavelength detector. The identity of a single peak in a mixture was confirmed by "spiking" the mixture with the component in question and noting an increase in the height of the particular peak.

Determination of capacity ratios

Retention times for all of the compounds in each mode were expressed as capacity ratios (k'). For the adsorption and normal partition modes, biphenyl was used as the reference for determination of t_0. Acetone was used in the reversed-phase partition mode.

RESULTS AND DISCUSSION

Normal adsorption chromatography

Capacity ratios for the series of substituted biphenyls have been determined in three different solvent systems. Generally the order of elution is that expected for normal adsorption chromatography[3]; *i.e.*, the retention times increase with an increase in polarity of the compound. The results obtained for this mode are summarized in Table I. The compounds are listed in the order of increasing k' values for solvent

TABLE I

CAPACITY RATIOS FOR SOME BIPHENYL DERIVATIVES ON A NORMAL ADSORPTION-TYPE COLUNN

Column, Partisil 10 PXS (4.6 mm I.D. × 25 cm). Conditions: UV detection at 254 nm, 0.5 a.u.f.s.; flow-rate, 70 ml/h; chart speed, 1 in./min; pressure, 600 p.s.i.; temperature, ambient. Solvent systems: (A) 45% hexane in 2-propanol–methylene chloride (30:70); (B) 3% methanol in chloroform; (C) 1% methanol in methylene chloride. A dash (—) indicates that the compound was not eluted in this system within 30 min or gave inconsistent results.

Compound	k'		
	Solvent system		
	A	*B*	*C*
Biphenyl	0.00	0.00	0.00
4-Nitrobiphenyl	0.00	0.00	0.00
3-Nitrobiphenyl	0.00	0.00	0.00
4,4′-Dinitrobiphenyl	0.00	0.00	0.00
4-Trifluoroacetamidobiphenyl	0.02	0.18	0.16
4-Trifluoroacetamido-4′-nitrobiphenyl	0.05	0.23	0.22
3-Acetoxy-4-acetamidobiphenyl	0.09	0.03	0.24
2-Aminobiphenyl	0.13	0.05	0.19
3-Hydroxy-4-nitrobiphenyl	0.35	0.03	0.07
2-Acetamidobiphenyl	0.39	0.28	0.97
3-Aminobiphenyl	0.59	0.34	0.48
4-Aminobiphenyl	0.74	0.43	0.52
4-Amino-4′-nitrobiphenyl	0.85	0.40	0.97
4-Trifluoroacetamido-4′-aminobiphenyl	0.94	0.88	1.01
3-Acetamidobiphenyl	1.29	0.57	1.19
3-Hydroxy-4-acetamidobiphenyl	1.33	0.95	1.30
4-Acetamidobiphenyl	1.59	0.75	1.34
N-Hydroxy-4-acetamidobiphenyl	1.65	0.84	1.42
4-Acetamido-4′-nitrobiphenyl	2.01	—	1.42
4,4′-Diaminobiphenyl (benzidine)	2.54	1.14	1.21
4,4′-Diacetamidobiphenyl	—	—	—
4-Acetamido-4′-aminobiphenyl	—	3.50	—
3-Hydroxy-4-aminobiphenyl	—	—	—

system A; the order of elution is not exactly the same for systems B and C. The reference compound biphenyl elutes in all three systems at or very near the solvent front. The elution order of the series of compounds is discussed for system A and the discussion may be generally applied to the other two systems.

In system A the two non-polar nitro compounds 3-nitrobiphenyl and 4-nitrobiphenyl, along with the symmetrical dinitro derivative 4,4′-dinitrobiphenyl, are not retained appreciably and elute virtually with the solvent front. Reduction of the nitro groups of the simple nitro derivatives results in longer retention times (larger k') for the resulting basic amino derivatives 3-aminobiphenyl and 4-aminobiphenyl. We observe that the order of elution increases in going from 2-amino to 3-amino to 4-aminobiphenyl. Furthermore, the 2-substituted amine is markedly separated from the other amines, as indicated by the observed k' values in Table I. Hanai and Walton[4] reported a similar observation with respect to the elution times for 2-chlorobiphenyl and 4-chlorobiphenyl on an ion-exchange column. They attributed the greatly reduced retention time for the 2-chloro derivative to a forcing of the two phenyl rings out of a common plane and thus a reduced π-electron overlap. The 3- and 4-amino compounds show less separation between themselves. Thus, there is a smaller effect on the retention time in moving the amino group from position 3 to 4 than in moving it from position two to position 3 or 4. As would be expected, monoreduction of the symmetrical dinitro compound 4,4′-dinitrobiphenyl to give the amino-nitro derivative 4-amino-4′-nitrobiphenyl results in an increase in the retention time over that for the parent compound. A second reduction to yield 4,4′-diaminobiphenyl (benzidine) results in a further increase in the observed retention time.

Acetylation of the three simple amines to yield the acetamides results in similar elution patterns. Position isomers elute in the same order as the amines, and the retention times of the acetamides 2-acetamido-, 3-acetamido-, and 4-acetamidobiphenyl are found to be greater than those of the corresponding amines. Again the marked separation of the 2-substituted derivative from the other two derivatives can be seen. In contrast, trifluoroacetylation of 4-aminobiphenyl to yield 4-trifluoroacetamidobiphenyl decreases retention time of the derivative compared to that of the parent amine. The compound 2-trifluoroacetamidobiphenyl (not included in Table I) has since been synthesized, and its retention time is also less than that of the parent amine.

The relative effects of substituents on retention time can also be seen in the symmetrically disubstituted biphenyl series. If the amino group of 4-amino-4′-nitrobiphenyl is trifluoroacetylated to form 4-trifluoroacetamido-4′-nitrobiphenyl, its retention time is shorter than that of the parent compound 4-amino-4′-nitrobiphenyl. The dominant effect of the polar amino group is virtually eliminated by this type of substitution. When the free amino group of the monoamine 4-amino-4′-nitrobiphenyl is acetylated to yield 4-acetamido-4′-nitrobiphenyl, the observed retention time is greater than that for the parent amine. Reduction of the nitro group of both of these derivatives results in an increase in retention time; *i.e.*, the reduced compound 4-trifluoroacetamido-4′-aminobiphenyl is retained to about the same extent as 4-amino-4′-nitrobiphenyl while 4-acetamido-4′-aminobiphenyl and the diacetylated compound 4,4′-diacetamidobiphenyl (diacetylbenzidine) are completely retained under the conditions employed.

A third group of compounds used in this study were substituted at positions

3 and 4 of one aromatic ring. Introduction of a hydroxyl group into the simple nitro derivative 4-nitrobiphenyl gave the *ortho* nitrophenol 3-hydroxy-4-nitrobiphenyl. The effect of the dominant hydroxyl group upon the retention time of the compound is not as great as one would expect ($k' = 0.35$). However, *ortho* nitrophenols may form intramolecular hydrogen bonds. This type of bonding should indeed reduce the retaining effect of the hydroxyl, and thus elution of the derivative is relatively fast. On the other hand, the *ortho* aminophenol 3-hydroxy-4-aminobiphenyl seems to be retained in this system. When both the 3-hydroxyl and 4-amino groups are acetylated, the resultant compound 3-acetoxy-4-acetamidobiphenyl elutes quite rapidly. Deesterification of the 3-hydroxyl to generate the amidophenol 3-hydroxy-4-acetamidobiphenyl leads to a marked increase in retention time. The N-hydroxylated compound N-hydroxy-4-acetamidobiphenyl is retained somewhat longer than the 3-hydroxy compound.

The other two solvent systems used for normal adsorption chromatography do not, as has been mentioned earlier, give exactly the same elution orders. However, the same general patterns for a given series of compounds are found with these solvent systems. Some differences in the elution order for closely eluting compounds may be explained by small changes in the composition of mixed solvents between runs.

The acidic compounds such as the hemisuccinyl derivatives are not reported in this phase of the study since they are retained strongly, but are discussed under reversed-phase chromatography.

Fig. 1 illustrates the separation of a mixture of randomly chosen amines and acetamides, as well as one of the nitro compounds, on the Partisil 10 column using gradient elution. The elution behavior of the individual components follows the

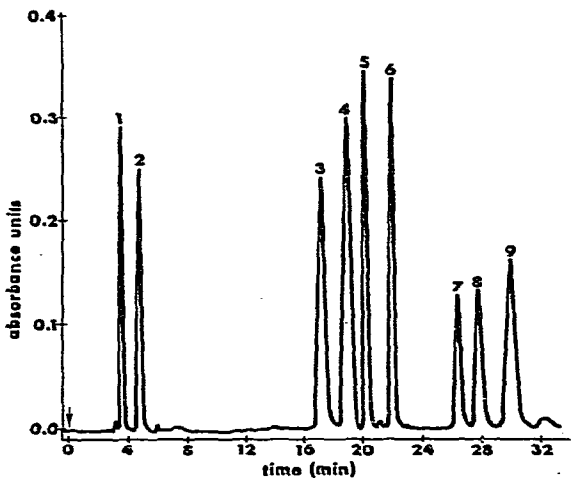

Fig. 1. HPLC of a mixture of biphenyl derivatives. 1 = 4-Nitrobiphenyl; 2 = 2-aminobiphenyl; 3 = 3-aminobiphenyl; 4 = 4-aminobiphenyl; 5 = 2-acetamidobiphenyl; 6 = 4-amino-4'-nitrobiphenyl; 7 = 3-acetamidobiphenyl; 8 = 4-acetamidobiphenyl; 9 = 4,4'-diaminobiphenyl (benzidine). Conditions: column, Partisil 10-PXS (4.6 mm I.D. × 25 cm); flow-rate, 60 ml/h; chart speed, 0.25 in./min; solvents: (A) hexane, (B) 2-propanol–methylene chloride (30:70), 15% initial B, increase at 2% B/min. UV detection at 254 nm, attenuation at 0.5 a.u.f.s.

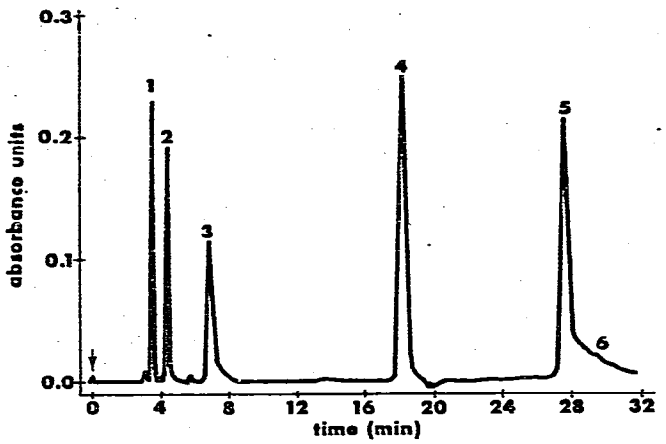

Fig. 2. HPLC of a mixture of biphenyl derivatives. 1 = 4-Nitrobiphenyl, 2 = 4-trifluoroacetamidobiphenyl, 3 = 3-acetoxy-4-acetamidobiphenyl, 4 = 4-aminobiphenyl, 5 = 4-acetamidobiphenyl 6 = 3-hydroxy-4-acetamidobiphenyl. Conditions are as in Fig. 1.

TABLE II

CAPACITY RATIOS FOR SOME BIPHENYL DERIVATIVES ON A "CYANO-TYPE" BONDED-PHASE NORMAL PARTITION COLUNN

Column, Partisil 10-PAC (4.6 mm I.D. × 25 cm). Conditions: UV detection at 254 nm, 0.5 a.u.f.s.; flow-rate, 80 ml/h; chart speed, 1 in./min; pressure, 400 p.s.i.; temperature, ambient. Solvent systems (A) 10% acetonitrile in methylene chloride; (B) 60% hexane in 2-propanol–methylene chloride (30:70); (C) 50% hexane in acetonitrile–methylene chloride (20:80). A dash (—) indicates that the compound did not elute under these conditions within 30 min or gave inconsistent results.

Compound	*k'*		
	Solvent system		
	A	*B*	*C*
Biphenyl	0.00	0.00	0.00
3-Nitrobiphenyl	0.00	0.05	0.13
3-Hydroxy-4-nitrobiphenyl	0.00	0.06	0.18
4,4'-Dinitrobiphenyl	0.02	0.15	0.33
4-Nitrobiphenyl	0.05	0.05	0.15
4-Trifluoroacetamido-4'-nitrobiphenyl	0.08	0.49	0.75
4-Trifluoroacetamidobiphenyl	0.10	0.28	0.36
2-Aminobiphenyl	0.17	0.18	0.38
3-Acetoxy-4-acetamidobiphenyl	0.36	0.25	—
4-Amino-4'-nitrobiphenyl	0.49	1.35	1.89
3-Aminobiphenyl	0.49	0.83	1.34
4-Aminobiphenyl	0.56	0.97	1.49
4-Trifluoroacetamido-4'-aminobiphenyl	1.23	—	2.26
2-Acetamidobiphenyl	1.43	0.64	2.36
3-Acetamidobiphenyl	3.27	2.84	—
4-Acetamidobiphenyl	3.51	3.13	—
4-Acetamido-4'-nitrobiphenyl	3.51	—	—
3-Hydroxy-4-acetamidobiphenyl	3.58	—	—
4,4'-Diaminobiphenyl (benzidine)	3.71	—	—
3-Hydroxy-4-aminobiphenyl	—	—	—
4-Acetamido-4'-aminobiphenyl	—	—	—
4,4'-Diacetamidobiphenyl	—	—	—
N-Hydroxy-4-acetamidobiphenyl	—	—	—

patterns described in the discussion above. The marked separation of the 2-substituted derivatives from the 3- and 4-derivatives, for example, is apparent. Separation of another mixture, which included two of the 3-substituted derivatives (Fig. 2), illustrates the significant effect esterification of a phenolic hydroxyl group (3-hydroxy-4-acetamidobiphenyl to form 3-acetoxy-4-acetamidobiphenyl) has upon reducing the polarity of the compound and its resulting retention time.

Normal-phase partition chromatography

Normal partition chromatography[5] on the bonded cyano (polar, CN) phase complements reversed-phase chromatography on the bonded octadecyl (non-polar) phase. We have determined the capacity ratios for most of the biphenyl derivatives used in this study employing three different "typical" solvent systems. The results for this mode of chromatography are given in Table II.

Solvent system A elutes most of the compounds. The order of elution, relative to the biphenyl marker, is generally the same as that observed for the normal adsorption mode (see Table I). Capacity ratios increase as the polarity of the compound increases.

Separation of a mixture of compounds on Partisil 10-PAC, a normal-phase column, under isocratic conditions is illustrated in Fig. 3. The pattern of elution is similar to that shown in Fig. 1. The significant feature is again the marked difference in retention time for 2-substituted biphenyls *versus* the 3- and 4-substituted biphenyls. Separation of the mixture was also carried out in the normal partition mode under gradient elution conditions (Fig. 4). One feature of note is the increase in retention time upon introduction of amino groups. The chromatograms also illustrate the effect on retention times caused by trifluoroacetylation and acetylation. The unidentified peak in the chromatogram is also seen in the solvent baseline.

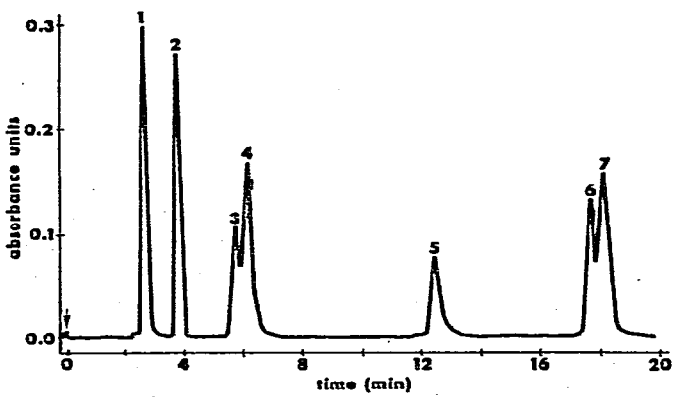

Fig. 3. HPLC of a mixture of biphenyl derivatives. 1 = 3-Nitrobiphenyl; 2 = 2-aminobiphenyl; 3 = 3-aminobiphenyl; 4 = 4-aminobiphenyl; 5 = 2-acetamidobiphenyl; 6 = 3-acetamidobiphenyl; 7 = 4-acetamidobiphenyl. Conditions: column, Partisil 10-PAC (4.6 mm × 25 cm); flow-rate, 80 ml/h; chart speed, 0.5 in./min; solvent, acetonitrile–methylene chloride (2:98); UV detection at 254 nm, attenuation at 0.5 a.u.f.s.

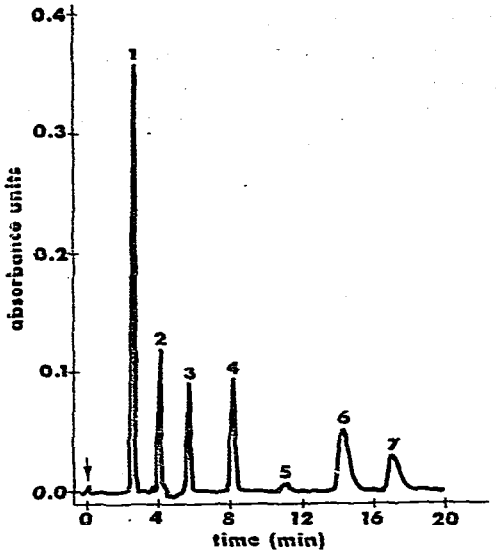

Fig. 4. HPLC of a mixture of biphenyl derivatives. 1 = Biphenyl; 2 = 4,4′-dinitrobiphenyl; 3 = 4-amino-4′-nitrobiphenyl; 4 = 4-trifluoroacetamido-4′-nitrobiphenyl; 5 = unknown; 6 = 4-acetamido-4′-nitrobiphenyl; 7 = benzidine. Conditions: column, see legend Fig. 3; flow-rate, 80 ml/h; chart speed, 0.25 in./min; solvent, (A) hexane, (B) 2-propanol–methylene chloride (30:70), initial 35% B, increase at 4% B/min; UV detection at 254 nm, attenuation at 0.5 a.u.f.s.

Reversed-phase partition chromatography

The capacity ratios for biphenyl derivatives which were examined in the reversed-phase mode are listed in Tables III and IV. The mechanisms involved in separations and selectivity on reversed-phase columns are discussed in detail elsewhere[6]. The compounds in Table III are arranged in order of increasing capacity ratios for the methanol–water system. As expected, polar compounds elute early and have low k' values. The discussion below is applicable for both the methanol–water and acetonitrile–water solvent systems. Some of the similarities and differences between the solvent systems are noted. Conversion of the 4-nitro compound 4-nitrobiphenyl and its 3-hydroxy derivative 3-hydroxy-4-nitrobiphenyl results in an increase in the retention time in the methanol–water system whereas the hydroxylated derivative elutes prior to the simple nitro compound in the acetonitrile–water system. Reduction of the nitrophenol to the aminophenol results in a decrease in retention time in the methanol–water system. This compound was not reported in the acetonitrile–water system because it displayed erratic behavior in this solvent system.

Other effects of a change in the solvent system used for elution can be demonstrated in several cases. In the methanol–water system the compounds 4-amino-4′-nitrobiphenyl and 4,4′-dinitrobiphenyl elute in that order. The trifluoroacetylated derivative 4-trifluoroacetamido-4′-nitrobiphenyl elutes later than either of those compounds. However, the acetylated compound 4-acetamido-4′-nitrobiphenyl elutes after 4-amino-4′-nitrobiphenyl and 4,4′-dinitrobiphenyl in the methanol–water system, but elutes before all three in the acetonitrile–water system.

Table III also contains the k' values for four biphenyl derivatives containing

TABLE III

CAPACITY RATIOS FOR A SERIES OF BIPHENYL DERIVATIVES ON A REVERSED-PHASE PARTITION COLUNN

Column, Partisil 10-ODS (4.6 mm I.D. × 25 cm). Values determined with reference to an acetone marker. Conditions: UV detection at 254 nm, 0.5 a.u.f.s., flow-rate, 90 ml/h; chart speed, 1 in./min; temperature, ambient.

Compound	*k'*	
	Solvent system	
	Methanol–water (70:30)	*Acetonitrile–water (30:70)*
2-Acetamidobiphenyl	0.55	0.45
$N^{4'}$-(4-Hydroxyphenethylaminohemisuccinyl)-4,4'-diaminobiphenyl	0.68	0.55
4-Acetamido-4'-aminobiphenyl	0.70	1.48
$N^{4'}$-(4-Hydroxyphenethylaminohemisuccinyl)-4-acetamido-4'-aminobiphenyl	0.81	0.34
4,4'-Diacetamidobiphenyl	0.84	0.30
4,4'-Diaminobiphenyl (benzidine)	0.88	1.24
4-Trifluoroacetamido-4'-aminobiphenyl	0.97	2.00
2-Aminobiphenyl	1.01	0.85
3-Hydroxy-4-acetamidobiphenyl	1.10	0.57
3-Hydroxy-4-aminobiphenyl	1.14	*
3-Aminobiphenyl	1.22	0.97
N^{4}-(4-Hydroxyphenethylaminohemisuccinyl)-4-aminobiphenyl	1.28	0.81
4-Aminobiphenyl	1.30	0.97
3-Acetamidobiphenyl	1.30	0.74
3-Acetoxy-4-acetamidobiphenyl	1.38	1.05
4-Acetamidobiphenyl	1.40	0.81
$N^{4'}$-(4-Hydroxyphenethylaminohemisuccinyl)-4-nitro-4'-aminobiphenyl	1.60	1.04
4-Amino-4'-nitrobiphenyl	1.65	1.40
4,4'-Dinitrobiphenyl	1.91	1.94
4-Nitrobiphenyl	2.05	2.19
4-Acetamido-4'-nitrobiphenyl	2.07	1.28
4-Trifluoroacetamidobiphenyl	2.22	1.42
3-Nitrobiphenyl	2.24	2.82
4-Trifluoroacetamido-4'-nitrobiphenyl	2.39	1.97
3-Hydroxy-4-nitrobiphenyl	2.72	1.85

* Gave inconsistent results

three aromatic rings, tyramine derivatives containing the 4-hydroxyphenethylamino-hemisuccinyl group which can be iodinated in the tyramine moiety. Since one ring of the biphenyl skeleton is similarly substituted in each of these four compounds the elution order in both solvent systems is influenced by the substituent in the other ring. We found that the polar amino and acetamido substituted compounds elute much earlier than the nitro or unsubstituted compounds.

The results for two other series deserve some mention. For the series consisting of benzidine, its monoacetylated (4-acetamido-4'-aminobiphenyl) derivative and its diacetylated (4,4-diacetamidobiphenyl) derivative, benzidine is retained longer than the diacetylated compound in both solvent systems. However, benzidine elutes faster

TABLE IV

CAPACITY RATIOS FOR SOME BIPHENYL HEMISUCCINAMIDES ON A REVERSED-PHASE COLUNN

Column: Partisil 10-ODS (4.5 mm I.D. × 25 cm). Conditions: UV detection at 254 nm, 0.5 a.u.f.s.; flow-rate, 90 ml/h; chart speed, 1 in./min; temperature, ambient.

Compound	*k'*	
	Solvent system	
	Methanol–water (70:30)*	*Acetonitrile–water* (30:70)*
4-Nitro-4'-hemisuccinamidobiphenyl	0.24	0.60
4-Trifluoroacetamido-4'-hemisuccinamidobiphenyl	0.25	0.60
4-Acetamido-4'-hemisuccinamidobiphenyl	0.74	0.36
4-Hemisuccinamidobiphenyl	1.24	0.90
4-Nitro-4'-hemisuccinamidobiphenyl	2.07	1.29

* With 0.1% glacial acetic acid added to the water.

than the monoacetylated derivative in the methanol–water system but elutes slower than the monoacetylated derivative in the acetonitrile–water system. The order of elution of the two nitro compounds is also anomalous, since the 3-isomer elutes after the 4-isomer.

Figs. 5–8 illustrate the applications of the k' data in Tables III and IV to the reversed-phase separation of mixtures of these derivatives. The five-component mixture (Fig. 5) represents a series of related reaction products. The first component 4,4'-dinitrobiphenyl was taken through a series of chemical reactions ending with conversion to the hemisuccinamide (peak 1).

The five hemisuccinamides were separated as illustrated in the chromatogram in Fig. 6. The early elution of the compound 4-amino-4'-hemisuccinamidobiphenyl (peak 2) seems inconsistent with the k' values in Table IV. However, the identity of the peak was confirmed by spiking the mixture.

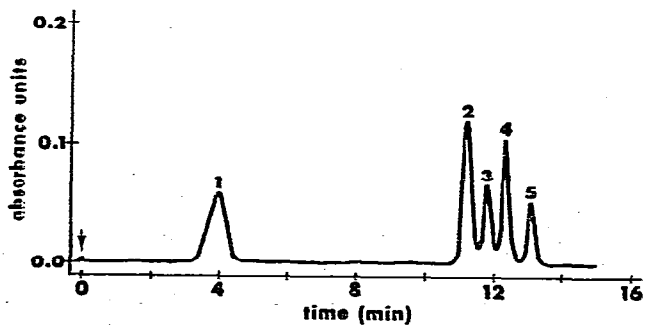

Fig. 5. HPLC of a mixture of biphenyl derivatives. 1 = 4-Trifluoroacetamido-4'-hemisuccinamidobiphenyl; 2 = 4-trifluoroacetamido-4'-aminobiphenyl; 3 = 4-amino-4'-nitrobiphenyl; 4 = 4,4'-dinitrobiphenyl; 5 = 4-trifluoroacetamido-4'-nitrobiphenyl. Conditions: column, Partisil 10-ODS (4.6 mm × 25 cm); flow-rate, 80 ml/h; chart speed, 0.5 in./min; solvents, (A) water, (B) methanol; 40% initial B, increase at 3% B/min.; UV detection at 290 nm, attenuation at 0.5 a.u.f.s.

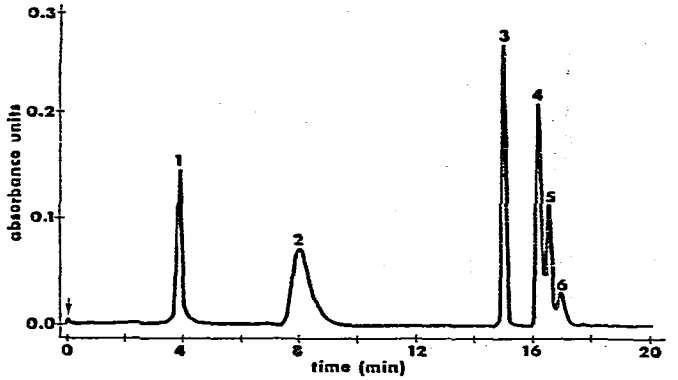

Fig. 6. HPLC of a mixture of biphenyl derivatives. 1 = Acetone; 2 = 4-amino-4′-hemisuccinamidobiphenyl; 3 = 4-acetamido-4′-hemisuccinamidobiphenyl; 4 = 4-trifluoroacetamido-4′-hemisuccinamidobiphenyl; 5 = 4-hemisuccinamidobiphenyl; 6 = 4-nitro-4′-hemisuccinamidobiphenyl. Conditions: column, see legend Fig. 5; flow-rate, 90 ml/h; chart speed 0.5 in./min; solvents, (A) water, 0.1% acetic acid, (B) acetonitrile, initial 20% B, increase at 1% B/min.; UV detection at 276 nm, attenuation at 0.5 a.u.f.s.

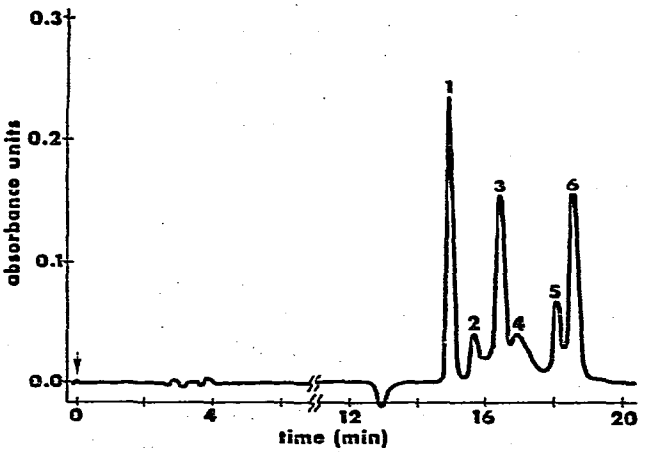

Fig. 7. HPLC of a mixture of biphenyl derivatives. 1 = 3-Hydroxy-4-acetamidobiphenyl; 2 = unknown; 3 = 3-acetoxy-4-acetamidobiphenyl; 4 = 3-hydroxy-4-aminobiphenyl; 5 = 3-hydroxy-4-nitrobiphenyl; 6 = 4-nitrobiphenyl. Conditions: column, see legend Fig. 5; solvents, (A) water, (B) acetonitrile, initial 25% B, increase at 2% B/min; UV detection at 276 nm, attenuation at 0.5 a.u.f.s.

The mixture illustrated in Fig. 7 is another series of related reaction products. The compound 4-nitrobiphenyl was taken through the sequence 3-hydroxy-4-nitrobiphenyl, 3-hydroxy-4-aminobiphenyl, 3-acetoxy-4-acetamidobiphenyl, and 3-hydroxy-4-acetamidobiphenyl. The influence of each change in the chemical nature of the biphenyl skeleton upon the polarity and retention time of the component can be seen. For example, deesterification of the 3-OH group in 3-acetoxy-4-acetamidobiphenyl yielded 3-hydroxy-4-acetamidobiphenyl which elutes from the column faster than the ester-amide.

Fig. 8A represents a mixture of related acetamides and trifluoroacetamides

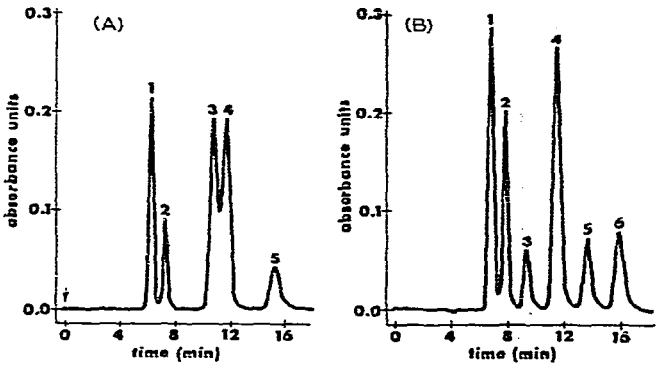

Fig. 8. Conditions: column, Partisil 10-ODS; flow-rate, 80 ml/h, chart speed, 0.25 in./min.; solvent, methanol–water (70:30), isocratic; UV detection at 254 nm, attenuation at 0.5 a.u.f.s. (A) 1 = 2-acetamidobiphenyl; 2 = 2-trifluoroacetamidobiphenyl; 3 = 3-acetamidobiphenyl; 4 = 4-acetamidobiphenyl; 5 = 4-trifluoroacetamidobiphenyl. (B) 1 = 4-acetamido-4′-aminobiphenyl; 2 = 4,4′-diacetamidobiphenyl; 3 = 4-trifluoroacetamido-4′-aminobiphenyl; 4 = 4-amino-4′-nitrobiphenyl; 5 = 4-acetamido-4′-nitrobiphenyl; 6 = 4-trifluoroacetamido-4′-nitrobiphenyl.

which are positional isomers. The 2-, 3-, and 4-acetamidobiphenyl derivatives elute according to the k' data given in Table III. A marked separation between the 2-substituted compound and the 3- and 4-substituted compounds is seen. The same pattern is observed for the trifluoroacetylated group. Finally, Fig. 8B is a chromatogram which illustrates the separation of a random mixture of derivatives. A noteworthy feature is the decreased retention time observed when a nitro group is converted to its corresponding amino derivative.

CONCLUSION

In this study we have presented the description of the behavior of a series of biphenyl derivatives in the three most commonly used modes of liquid chromatography. The conditions and choice of solvent systems were based upon our own experiences with these compounds. We do not suggest that these are the only conditions or solvents available which will produce the desired separations. However, the results of this study can serve as a guide which may be of value to those who are interested in the chromatography of these types of compounds.

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