of the pyrrole fragment are:

$$pI_{50} = 105.773 + 503.033\,S_9^{(e)} + 27.992\,Q_9 - 183.106\,S_{13}^{(e)} + 8.801\,Q_{13} - 62.903\,S_{11}^{(e)} - 176.198\,Q_{11} - 46.928\,Q_{12} - 63.207\,Q_{10} \quad \text{(Eq. A2)}$$

with $RSD = 0.10$, $R = 0.994$, $MSD = 0.028$, $F_{8,4} = 41.67$, and $p < 0.005$. These correlations are poorer than the one found for the indole group.

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New Biphenyl Derivatives II: 1-(4-Biphenylyl)-1-hydroxy-2-aminoethanes and 1-(4-Biphenylyl)-1-chloro-2-aminoethanes as Potential β-Adrenoceptor Blocking Agents

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Abstract □ Series of 1-(4-biphenylyl)-1-hydroxy-2-aminoethanes and 1-(4-biphenylyl)-1-chloro-2-aminoethanes were synthesized. Newly developed reaction conditions for aryl aminomethyl ketone reduction and reductive alkylation, using sodium borohydride, are described. The prepared compounds were examined for adrenergic blocking activity on an anesthetized dog blood pressure preparation and on isolated toad hearts. β-Adrenergic blockade was investigated using isoproterenol as the agonist. The benzylamino and cyclohexylamino analogs exhibited marked β-adrenoceptor blocking activity, for which the latter derivatives were more potent.

Keyphrases □ β-Adrenergic blocking activity—biphenyl derivatives, synthesis, structure–activity relationships □ Biphenyl derivatives, various—synthesized, evaluated for β-adrenergic blocking activity, structure–activity relationships □ Structure–activity relationships—biphenyl derivatives, β-adrenergic blocking activity

Optimum β-adrenoceptor blockade occurs when 1-phenyl-1-hydroxy-2-aminoethane structures (I) bear certain substituents at the phenyl 4- or 3,4-position and an isopropyl grouping on the amine head (1). 1-(3,4-Dichlorophenyl)-1-hydroxy-2-isopropylaminoethane (2) (II), 1-(2-naphthyl)-1-hydroxy-2-isopropylaminoethane (pronethalol) (3, 4) (III), and 1-(4-nitrophenyl)-1-hydroxy-2-isopropylaminoethane (5) (IV) are well-known examples.

In vitro hydrolysis of 1-aryl-1-chloro-2-aminoethanes to 1-aryl-1-hydroxy-2-aminoethanes was used to prepare a series of 1-chloro-2-aminoethanes related to III (6, 7).

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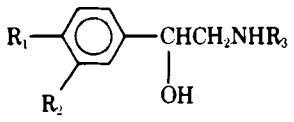


I: $R_1 = R_2 = R_3 = H$
II: $R_1 = R_2 = Cl$, $R_3 = CH(CH_3)_2$
III: R_1 and R_2 = parts in naphthalene ring, $R_3 = CH(CH_3)_2$
IV: $R_1 = NO_2$, $R_2 = H$, $R_3 = CH(CH_3)_2$

Their β-adrenergic blockade was concordant with that given by their corresponding 1-hydroxy-2-aminoethanes, thus proving the presumed *in vivo* hydrolysis. β-Adrenergic blockade studies on compounds possessing other phenyl or substituted phenyl groupings on the I aromatic function were unreported. Interest in new biphenyl derivatives (8) prompted the synthesis and subsequent pharmacological testing of some 1-biphenylyl-2-aminoethane (V) derivatives. The present investigation describes the preparation and preliminary biological evaluation of 1-(4-biphenylyl)-1-hydroxy-2-aminoethanes (VI) and their corresponding 1-chloro analogs (VII).

RESULTS AND DISCUSSION

Chemistry—Halohydrin amination and aminomethyl ketone reduction and/or reductive alkylation using any previously reported conditions (9), were unreliable for production of VI. The present work is confined to the reduction and reductive alkylation approaches using sodium borohydride. In this respect, the observed differences in reactivities for the various aryl aminomethyl ketones is believed to result from a shift in the keto–enol equilibrium that should exist at pH 8 (Scheme I) and which might be due to the aromatic function resonance contribution.

The most pronounced effect was given by the biphenylyl radical; its aminomethyl ketone could exist almost entirely as the enol (X). The latter would be converted completely to the enolate anion (XI) at pH > 8, leading to decomposition demonstrated by a darkening in the solution color. The predominance of X at pH 8 was substantiated by primary aminomethyl ketone stability at such a pH and by the failure of 4-biphenylyl aminomethyl ketone hydrochloride (XIII) acetylation at pH > 8.

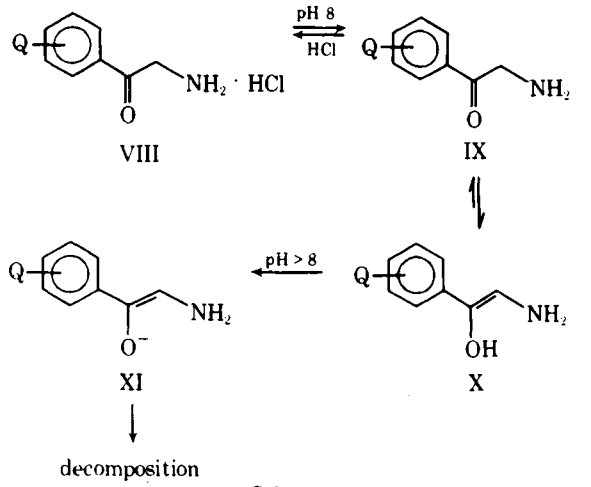
These observations also were supported by the similarity in chemical behavior between derivatives of 2-aminophenol and X. The most important properties were the ability of both groups to undergo diazotization (10) and to give the characteristic phenol ferric chloride test. The enol form of the secondary aminomethyl ketone derivative, 4-biphenylyl isopropylaminomethyl ketone (XVI*a*), solubilized in caustic alkali solutions without appreciable decomposition. Moreover, a stable enol form for the secondary amino derivative 4-biphenylyl anilinomethyl ketone (XVI*b*), namely 1-(4-biphenylyl)-1-hydroxy-2-anilinoethene (XVII), could be isolated.

Sodium borohydride ketonic group reduction *via* hydride-ion transfer followed by exchange with the hydroxylic solvent requires reactive borohydride anion production through sodium borohydride alkalinity (11). Such alkalinity ordinarily would not interfere with the reduction if the equilibrium (Scheme I) were shifted toward the keto form (IX); otherwise, an increase in the enol form (X) equilibrium shift would effect the conversion to the enolate anion (XI), resulting in decomposition rather than reduction of the aminomethyl ketone. The latter effect was prominent with XIII due to its relatively strong acidic enol form.

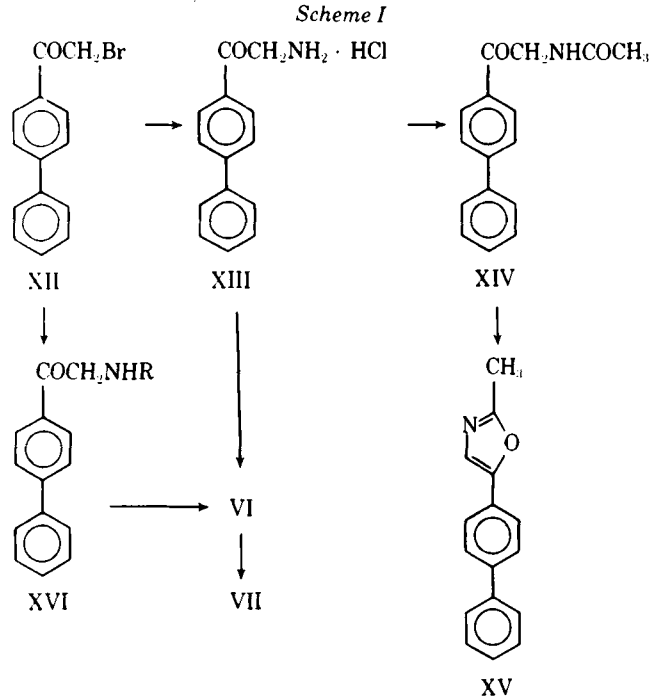
To maintain the medium pH at ≃ 8, polyhydroxy compounds, *e.g.*, glycerol and mannitol, were incorporated into the reaction mixture. Their ability to react with the liberated boric acid from sodium borohydride produced stronger acids. The best reduction medium was methanol–glycerol–water. For reductive alkylation, the produced glyceroboric acid could also catalyze the initial Schiff-base formation step with aldehydes or ketones. Water provided H^+ and OH^- ions necessary for the reaction. The described reductive alkylation procedure was applied successfully with aliphatic and aromatic aldehydes as well as with aliphatic and alicyclic ketones.

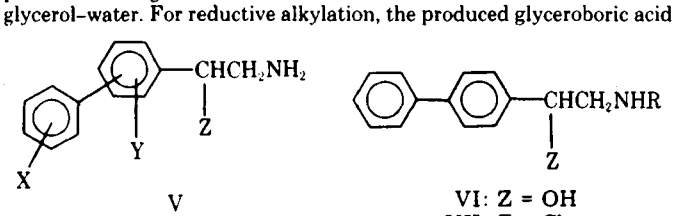
The synthesis of VI and VII (Table I) is outlined in Scheme II. The required 4-biphenylylaminomethyl ketones (XVI) were obtained by 4-biphenylylbromomethyl ketone amination (XII) (12). The primary aminomethyl ketone (XIII) key intermediate was prepared by reacting XII with hexamine in carbon tetrachloride followed by hydrolysis of the separated adduct. Treatment of a cooled aqueous XIII suspension with acetic anhydride and sodium acetate afforded the 4-biphenylylacetamidomethyl ketone (XIV).

The unexpected strong liability for dehydration of XIV to 5-(4-biphenylyl)-2-methyloxazole (XV) was seen upon treatment with aluminum isopropoxide in isopropanol under the usual Meerwein–Ponndorf–Verley reduction conditions. Unequivocal synthesis of XV was achieved by refluxing a XIV solution in acetic anhydride. The described reduction and reductive alkylation processes were applied for the preparation of the different 1-(4-biphenylyl)-1-hydroxy-2-aminoethanes (VI)



Scheme I

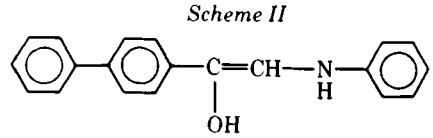




V

VI: Z = OH
VII: Z = Cl

Scheme II



XVII

Table I—1-(4-Biphenylyl)-1-hydroxy-2-aminoethanes (VI) and 1-(4-Biphenylyl)-1-chloro-2-aminoethanes (VII)

Compound	Z	R	Method	Yield, %	Melting Point[a]	Formula	Analysis, %	Calc.	Found
VI*a*	OH	H	C	90	234–236° dec.	$C_{14}H_{16}ClNO$[b,c]	C	67.3	67.2
							H	6.5	6.6
							Cl	14.2	14.2
							N	5.6	5.6
VI*b*	OH	$CH(CH_3)_2$	C, D[d]	89, 84	200–202°[e]	$C_{17}H_{22}ClNO$[b,f]	C	70.0	69.9
							H	7.6	7.2
							Cl	12.2	12.4
							N	4.8	4.6
VI*c*	OH	$CH_2CH(CH_3)_2$	D[g]	81	223–225°	$C_{18}H_{24}ClNO$[b]	C	70.7	70.4
							H	7.9	7.7
							Cl	11.6	11.2
							N	4.6	5.0
VI*d*	OH	$CH(CH_3)CH_2CH_3$	D[h]	72	212–214°	$C_{18}H_{24}BrNO$[i]	C	61.7	61.7
							H	6.9	6.6
							Br	22.8	23.2
							N	4.0	3.8
VI*e*	OH	$CH_2C_6H_5$	D[j]	68	245–247°	$C_{21}H_{22}ClNO$[b]	C	74.2	74.4
							H	6.5	6.4
							Cl	10.4	10.6
							N	4.1	4.5
VI*f*	OH	C_6H_{11}[k]	D[l]	74	245–247° dec.	$C_{20}H_{26}ClNO$[b]	C	72.4	72.0
							H	7.9	7.8
							Cl	10.7	10.5
							N	4.2	4.5
VII*a*	Cl	H	E	68	195–196° dec.	$C_{14}H_{15}Cl_2N$[b]	C	62.7	62.3
							H	5.6	5.2
							Cl	26.4	26.4
							N	5.2	5.0
VII*b*	Cl	$CH(CH_3)_2$	E	73	209–211°	$C_{17}H_{21}Cl_2N$[b]	C	65.8	65.9
							H	6.8	6.5
							Cl	22.9	23.1
							N	4.5	4.5
VII*c*	Cl	$CH_2CH(CH_3)_2$	E	70	173–175° dec.	$C_{18}H_{23}Cl_2N$[b]	C	66.7	67.0
							H	7.1	7.1
							Cl	21.9	22.2
							N	4.3	4.6
VII*d*	Cl	$CH(CH_3)CH_2CH_3$	E	65	170–172° dec.	$C_{18}H_{23}Cl_2N$[b]	C	66.7	66.6
							H	7.1	7.1
							Cl	21.9	22.2
							N	4.3	4.0
VII*e*	Cl	$CH_2C_6H_5$	E	54	174–176° dec.	$C_{21}H_{21}Cl_2N$[b]	C	70.4	70.2
							H	5.9	6.0
							Cl	19.8	20.2
							N	3.9	3.5
VII*f*	Cl	C_6H_{11}[k]	E	78	199–201° dec.	$C_{20}H_{25}Cl_2N$[b]	C	68.6	68.4
							H	7.2	7.5
							Cl	20.2	19.9
							N	4.0	3.8

[a] Recrystallization was done from methanol–ethyl acetate. [b] Hydrochloride. [c] Lit. (16) mp 209–211° dec. [d] Acetone was used. [e] Admixture of the products obtained by Methods C and D showed no melting-point depression. [f] Lit. (16) mp 175–177°. [g] Isobutyraldehyde was used. [h] Methyl ethyl ketone was used. [i] Hydrobromide. [j] Benzaldehyde was used. [k] Cyclohexyl. [l] Cyclohexanone was used.

from XIII and XVI*a*. The 1-(4-biphenylyl)-1-chloro-2-aminoethanes (VII) were produced by reacting the appropriate VI with thionyl chloride, either in chloroform or benzene (6). Structures were confirmed by IR and PMR studies.

Pharmacology—Compounds VI*a*–VI*f* and VII*a*–VII*f* were tested for cardiovascular activity on an anesthetized dog blood pressure preparation and isolated toad hearts. β-Adrenoceptor blocking was investigated using isoproterenol as the agonist.

For the anesthetized dog blood pressure method (13), anesthesia was induced by ether inhalation and was maintained by intravenous chloralose–urethan. The compounds were dissolved in water and injected into the femoral vein, and blood pressure was recorded from the carotid artery. The dose levels were 3×10^{-5} and 15×10^{-4} mole/kg. Each biphenylylhydroxyaminoethane and its chloro analog (VI*b* and VII*b*) gave the same type and magnitude of biological activity. No hypotensive effect was observed for the primary amine derivatives (VI*a* and VII*a*).

Table II compares the hypotensive effects obtained for VI*b*–VI*f* and VII*b*–VII*f*. With the exception of the cyclohexylamino derivatives (VI*f* and VII*f*), the hypotensive effects for all other *N*-substituted analogs (VI*b*–VI*e* and VII*b*–VII*e*) were not antagonized by pronethalol. On the other hand, they antagonized the epinephrine agonist effect in the order isopropylamino (VI*b* and VII*b*) > benzylamino (VI*e* and VII*e*) > *sec*-butylamino (VI*c* and VII*c*) > isobutylamino (VI*d* and VII*d*) derivatives.

Compounds VI*b* and VII*b* did not display any isoproterenol antagonism. The other *N*-substituted analogs (VI*c*–VI*f* and VII*c*–VII*f*) exhibited isoproterenol antagonism of the order cyclohexylamino (VI*f* and VII*f*) > benzylamino (VI*e* and VII*e*) ≫ *sec*-butylamino (VI*c* and VII*c*) > isobutylamino (VI*d* and VII*d*) derivatives. In all cases, gradual β-blockade onset was observed. Table II indicates that VI*b* and VII*b* might possess α-adrenoceptor blocking activity whereas VI*e*, VI*f*, VII*e*, and VII*f* revealed significant β-adrenoceptor blockades.

For the isolated toad heart method (14), the doses were 3×10^{-5} and 3×10^{-4} mole/ml. The experiments showed hypotensive effects for all tested compounds. This effect could not be blocked by atropine (3×10^{-5} mole/ml) and thus indicated direct myocardial depression. Isoproterenol antagonism, *i.e.*, β-blockade, was only exhibited by VI*e* and VII*e* and VI*f* and VII*f* analogs. The most pronounced effect was produced by VI*f* and VII*f*.

EXPERIMENTAL[1]

4-Biphenylylaminomethyl Ketone Hydrochloride (XIII)—A hot suspension of hexamine (9.8 g, 0.07 mole) in carbon tetrachloride (100 ml) was gradually added to a hot stirred XII solution (13.8 g, 0.05 mole) in carbon tetrachloride (50 ml). The reaction mixture was stirred for 1.5

[1] IR spectra were determined on a Unicam SP 200 spectrophotometer with Nujol mulls. PMR spectra were determined on a Perkin-Elmer R 12 spectrometer with tetramethylsilane as the internal standard. Melting points were determined in open glass capillaries and are uncorrected. Microanalyses were performed by the Microanalytical Unit, Faculty of Science, University of Cairo, Cairo, Egypt.

Table II—Comparison of the Hypotensive Effects[a] of VIb–VIf and VIIb–VIIf Using the Anesthetized Dog Blood Pressure Method

Compound[b,c]	Mean Percent Reduction[d] of Agonist Response ± SE: Epinephrine[b] Antagonism	Isoproterenol[e] Antagonism
VIb	23.33 ± 0.83	—
VIc	11.70 ± 1.18	10.83 ± 0.94
VId	10.00 ± 1.44	6.66 ± 0.83
VIe	13.33 ± 0.83	49.33 ± 2.24
VIf[f]	—	67.50 ± 1.44
VIIb	21.70 ± 1.67	—
VIIc	10.83 ± 0.83	10.00 ± 1.03
VIId	9.16 ± 1.66	6.00 ± 0.76
VIIe	12.50 ± 1.44	47.50 ± 1.44
VIIf[f]	—	64.17 ± 0.83

[a] Not antagonized by pronethalol (3×10^{-5} mole/kg). [b] The dose used was 15×10^{-4} mole/kg. [c] Aqueous solutions were used. [d] Average of three experiments. [e] The dose was 3×10^{-5} mole/kg. [f] The hypotensive effect was antagonized by pronethalol (3×10^{-5} mole/kg).

hr. It was then refluxed for 1 hr, cooled, diluted with acetone (50 ml), and filtered. The crude adduct, 4-biphenylylhexamethylene tetraammonium methyl ketone bromide, was triturated with cold ethanol, filtered, and dried in air. The yield was 17.5 g (84%), mp 162°.

Hydrolysis of the adduct (12.5 g, 0.03 mole) was affected by brief heating on a water bath with ethanol (50 ml) and concentrated hydrochloric acid (9 ml). The reaction mixture was treated with 10% HCl dropwise until turbidity appeared. It was then decolorized by boiling with charcoal, filtered, concentrated *in vacuo*, and left to crystallize. The product was recrystallized from 10% HCl to yield 5.6 g (74%) of product, mp > 300°; IR: 3000 (b, NH_3^+) and 1680 (C=O) cm^{-1}.

Anal.—Calc. for $C_{14}H_{14}ClNO$: C, 67.8; H, 5.7; Cl, 14.4; N, 5.6. Found: C, 67.4; H, 5.8; Cl, 14.0; N, 5.3.

4-Biphenylylacetamidomethyl Ketone (XIV)—Acetic anhydride (3.1 g, 0.03 mole) was added, in one portion, to a XIII suspension (3.7 g, 0.015 mole) in water (25 ml) and crushed ice (50 g). The mixture was treated dropwise, while being stirred, with a sodium acetate solution (5 g, 0.06 mole) in water (20 ml) at a rate such that the temperature was not allowed to rise above 5°. The reaction mixture was allowed to reach room temperature and was treated with 10% HCl (20 ml).

The product was filtered, washed with water several times, dried, and recrystallized from benzene–petroleum ether (bp 40–60°). The yield was 2.6 g (71%), mp 164–166° [lit. (15) mp 151°]; IR: 3400 (NH), 1685 (C=O), and 1645, 1550, and 1295 (amide I, II, and III, respectively) cm^{-1}.

Anal.—Calc. for $C_{16}H_{15}NO_2$: C, 75.9; H, 6.0; N, 5.4. Found: C, 75.7; H, 5.9; N, 5.3.

5-(4-Biphenylyl)-2-methyloxazole (XV)—*Method A*—Under strictly anhydrous conditions, a stirred mixture of XIV (1.2 g, 0.005 mole) and an aluminum isopropoxide solution (1.5 g, 0.0075 mole) in dry isopropanol (50 ml) was heated at 90° in a 100-ml flat-bottom flask. The flask was fitted with a reflux air condenser and an upper distillation head, which was attached to a downside condenser connected to a water pump *via* a buchner receiver. The reaction mixture was distilled slowly, at a rate not exceeding 7–10 drops/min at 40 mm Hg. No acetone was detected in the distillate collected during 1.5 hr.

The residue was cooled, and the remaining complex was treated with crushed ice. The mixture was extracted with benzene, washed with water, dried over anhydrous sodium sulfate, and filtered; then the solvent was removed *in vacuo*. The product was recrystallized from benzene–petroleum ether (bp 40–60°) to yield 0.9 g (76%), mp 167°.

Method B—A mixture of XIV (1.2 g, 0.005 mole) and acetic anhydride (20 g, 0.2 mole) was refluxed for 30 min. The reaction mixture was concentrated *in vacuo* to a small volume and then treated with crushed ice. The medium was made alkaline with sodium carbonate, and the product was filtered, washed with water, dried, and recrystallized from benzene–petroleum ether (bp 40–60°). The yield was 0.95 g (80%), mp 167°.

Admixture of the products obtained by Methods A and B showed no melting-point depression; IR: 1670 (C=N) and 1240 (aromatic C–O–C) cm^{-1}.

Anal.—Calc. for $C_{16}H_{13}NO$: C, 81.7; H, 5.6; N, 6.0. Found: C, 82.0; H, 5.2; N, 6.0.

4-Biphenylylisopropylaminomethyl Ketone (XVIa) Hydrochloride—A solution of isopropylamine (0.59 g, 0.01 mole) in carbon tetrachloride (20 ml) was gradually added, with stirring, to a solution of XII (2.8 g, 0.01 mole) in carbon tetrachloride (20 ml) at 0°. The stirring was maintained for an additional 2 hr. The reaction mixture was refluxed for 30 min, cooled, and extracted with 5 *N* NaOH. The alkaline extract was washed twice with benzene and made strongly acidic with concentrated hydrochloric acid. The product was filtered, washed with acetone, and recrystallized from methanol–ethyl acetate. The yield was 0.5 g (17%); IR: 2750 (NH_2^+) and 1680 (C=O) cm^{-1}.

Anal.—Calc. for $C_{17}H_{20}ClNO$: C, 70.4; H, 6.9; Cl, 12.2; N, 4.9. Found: C, 70.2; H, 6.6; Cl, 12.5; N, 4.5.

4-Biphenylylanilinomethyl Ketone (XVIb) and 1-(4-Biphenylyl)-1-hydroxy-2-anilinoethene (XVII)—A solution of aniline (0.93 g, 0.01 mole) in carbon tetrachloride (20 ml) was gradually added, with stirring, to a solution of XII (2.8 g, 0.01 mole) in carbon tetrachloride (20 ml) at 0°. The stirring was maintained for an additional 2 hr. The reaction mixture was refluxed for 30 min, cooled, and shaken with 5 *N* NaOH (20 ml). The carbon tetrachloride layer was washed twice with water and dried over anhydrous sodium sulfate, and the solvent was removed *in vacuo*.

The residue was boiled with methanol and filtered. From the methanolic extract, XVIb was crystallized out as colorless needles to yield 0.3 g (10%), mp 125–128°. The methanol-insoluble fraction was recrystallized from acetone as bright-yellow flakes (XVII) to yield 2.1 g (70%), mp 185–186°. The colorless XVIb changed to the yellow XVII within 84 hr at room temperature; IR (XVIb): 3390 (C=O) cm^{-1}; IR (XVII): 3410 (NH) and 3100 (b, OH) cm^{-1}; PMR (XVII, $CDCl_3$): δ 4.60 (s, 1H, OH), 4.90 (b, 1H, NH), 7.20 (m, 1H, CH), and 8.10 (m, 14H, aromatic) ppm.

Anal.—Calc. for $C_{20}H_{17}NO$: C, 83.6; H, 6.0; N, 4.9. Found: C, 83.3; H, 6.1; N, 4.8.

1-(4-Biphenylyl)-1-hydroxy-2-aminoethanes—*Method C (VIa and VIb)*—Sodium borohydride (0.95 g, 0.025 mole) was added in portions during 1.5 hr to a 0° cooled and stirred mixture of the appropriate 4-biphenylylaminomethyl ketone (XIII and XVIa, 0.01 mole), glycerol (23 g, 0.25 mole), water (2 ml), and methanol (100 ml). Cooling and stirring were maintained for 3 hr, and the reaction mixture was stirred at room temperature for a further 15 hr. It was then treated with concentrated hydrochloric acid dropwise until just acidic to litmus, concentrated *in vacuo*, and made strongly alkaline with 5 *N* NaOH. The product was extracted with benzene, washed twice with water, dried over anhydrous sodium sulfate, and filtered. Dry hydrogen chloride gas was bubbled into the filtrate, and the separated salt was collected and recrystallized.

Method D (VIb–VIf)—Sodium borohydride (1.4 g, 0.036 mole) was added in portions during 1.5 hr to a 0° cooled and stirred mixture of XIII (2.75 g, 0.01 mole), the proper aldehyde or ketone (0.1 mole), glycerol (33.2 g, 0.36 mole), water (2 ml), and methanol (100 ml). The reaction mixture was worked up as detailed under *Method C*, and the appropriate salt was prepared in the usual manner and recrystallized; IR (VIa–VIf): 3350–3300 (OH) and 2500–2400 (with several splits, NH_2^+) cm^{-1}; PMR (VIb, CD_3OD–$CDCl_3$): δ 1.35 (d, 6H, $2CH_3$ of isopropyl), 3.10 (m, 2H, methylene), 3.40 (m, 1H, CH of isopropyl), 5.15 (m, 1H, CH), 6.85 (b, 1H, OH), 7.45 (m, 9H, aromatic), and 8.15 (b, 1H, NH) ppm.

1-(4-Biphenylyl)-1-chloro-2-aminoethanes (VII)—*Method E*—Thionyl chloride (2.4 g, 0.02 mole) was added dropwise to a stirred suspension of the proper VI (0.01 mole) in either chloroform or benzene (75 ml). The reaction mixture was refluxed for 1.5 hr, and the solvent was removed *in vacuo*. The residue was recrystallized.

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Modified USP Assay for Simultaneous Determination of Aspirin and Nonaspirin Salicylates in Aspirin and Buffered Aspirin Tablets

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Abstract □ Modified USP procedures are described for the simultaneous determination of nonaspirin salicylates and aspirin in aspirin and buffered aspirin tablets. The existing USP procedures are not stability indicating for intact aspirin when significant levels of nonaspirin salicylates are present, as is often the case in short-term, high temperature stability programs. The modified procedures yield considerably shorter analysis times and stability-indicating assays for intact aspirin without the need for sophisticated equipment other than that presently required by USP XIX.

Keyphrases □ Aspirin—analysis, modified USP method, liquid chromatography, spectrophotometry, stability, buffered and unbuffered tablets □ Liquid chromatography—analysis, aspirin in buffered and unbuffered tablets, modified USP method, stability □ Analgesics—aspirin, analysis, buffered and unbuffered tablets, modified USP method, liquid chromatography, spectrophotometry, stability

The USP methods (1) for the quantitative determination of aspirin and nonaspirin salicylates in aspirin and buffered aspirin tablets are time consuming and nonspecific for evaluation of intact aspirin stability. The aspirin assay is based mainly on the work of Levine (2). A sample preparation in chloroform is passed through a sodium bicarbonate-treated, infusorial earth-packed column, which separates aspirin from tablet excipients. Following its elution, the aspirin is quantitated spectrophotometrically.

Although the method is reliable for production control, its accuracy as a stability-indicating assay is questionable because it does not isolate aspirin from its degradation product salicylic acid. Since the bicarbonate column concurrently traps both aspirin and salicylic acid, the result of their simultaneous elution is a positive interference in the aspirin UV absorption at 280 nm.

Another weakness in the USP method is that separate procedures are required for the aspirin and nonaspirin salicylates assays; hence, the complete analysis of aspirin tablets is quite lengthy. The USP nonaspirin salicylates assay is based on several studies (3–7). A sample is triturated in chloroform in the presence of citric acid monohydrate. Insoluble salts of aspirin or salicylic acid, if present, are converted to chloroform-extractable free acids by hydrochloric acid. Finally, the salicylic acid is separated from aspirin using an infusorial earth column treated with ferric chloride and urea, which complexes salicylic acid. The salicylic acid is eluted and quantitated spectrophotometrically.

This paper reports a modified USP method that simultaneously determined aspirin and nonaspirin salicylates in aspirin and buffered aspirin dosage forms. Complexing the nonaspirin salicylates on a ferric chloride–urea infusorial earth column and collecting the intact aspirin eluate achieved effective separation. Aspirin in the eluate was determined spectrophotometrically. The complexed nonaspirin salicylates were eluted and likewise determined spectrophotometrically. The results of this modification were a considerably shorter analysis time, a stability-indicating assay for aspirin, and a method that could be performed without sophisticated equipment other than that already required by the USP.

EXPERIMENTAL

Reagents and Chemicals—All chemicals and reagents were USP or ACS grade and were used without further purification.

Aspirin Tablet Assay—*Chromatographic Column*—A column was packed as described under the nonaspirin salicylates procedure in the USP. It was washed with a 25-ml portion of chloroform.

Aspirin Standard Preparation—About 50 mg of USP aspirin reference standard, accurately weighed, was dissolved in glacial acetic acid–chloroform (1:99) and diluted to 50 ml in a volumetric flask. A 5.0-ml portion was transferred to a 100-ml volumetric flask containing 2.0 ml of methanol, diluted to volume with glacial acetic acid–chloroform, and mixed.

Salicylic Acid Standard Preparation—A suitable, accurately weighed, quantity of salicylic acid was dissolved in chloroform to obtain a solution containing 30 μg of salicylic acid/ml. A 5.0-ml portion was transferred

Table I—Recovery of Added Aspirin (50.00 mg) by USP Procedure

Salicylic Acid Added, mg	Aspirin Recovered, mg	Recovery, %
0.00	50.00	100.0
0.75	50.35	100.7
1.50	50.95	101.9
2.25	51.50	103.0
3.00	52.10	104.2

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