Journal of Medicinal and Pharmaceutical Chemistry

VOL. 2, No. 1 (1960)

Antiviral Compounds—II. Reaction of 4-Biphenylglyoxal with *p*-Aminobenzoic Acid and Related Structures

G. CAVALLINI, E. MASSARANI and D. NARDI, *Medicinal Chemistry Research Laboratories, Vister-Casatenovo Brianza (Como), Italy*

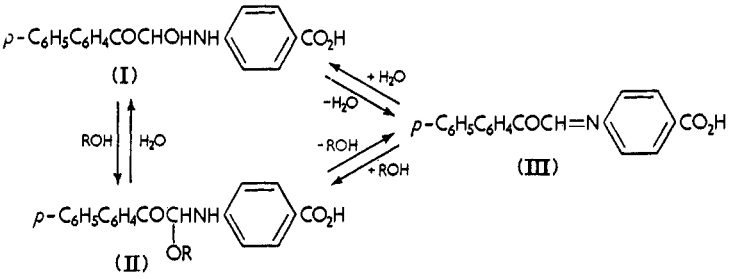
Introduction

In a previous paper[1] the synthesis of 4-biphenylglyoxal has been reported and its pronounced antiviral activity has been recorded. Since this compound was not well absorbed when administered orally to animals, some derivatives were prepared among which substances with a very favourable therapeutic ratio were discovered. As an approach to such derivatives, condensation products of 4-biphenylglyoxal were chosen which contained weak connecting bonds so that their structure might favour absorption and, at the same time, lessen the toxicity of the parent compound.

The most active and best tolerated derivative was found in the condensation product of *p*-aminobenzoic acid and 4-biphenylglyoxal (Table IV). On the basis of the observations of Musante and Parrini[2] one would have expected to obtain a derivative of structure (III), but the elementary analysis of the product was not in accord with such a formula. Acidimetric titration of this substance gave an equivalent weight greater than expected, and the infrared spectrum showed the presence of a N—H band (ν_{KBr}3408 cm^{-1}), and a C—O ether band (ν_{KBr} 1058 cm^{-1}). Since the reaction had taken place in ethanol, 4-biphenylglyoxal could have reacted as an hemiacetal. This assumption was confirmed by a Zeisel determination which demonstrated the presence of one ethoxyl group, and thus it became possible to propose structure (II) ($R = C_2H_5$) for the condensation product.

When heated at 140° at 15 mm, the substances changed to a yellow product having the same decomposition point, but the

material no longer contained an ethoxyl group. The loss in chemical composition equalled one molecule of ethanol, and the



N—H and C—O ether bands had disappeared from the infrared spectrum. The ethyl alcohol liberated in the reaction was col-

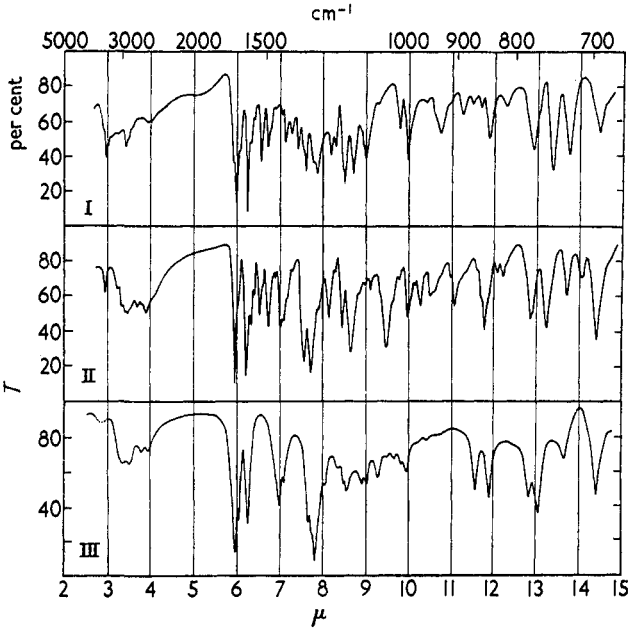


Fig. 1. Infrared spectra (KBr) of (I), (II), (III).

lected in a gas cell and characterized by means of its infrared spectrum. From these observations, and the elementary analysis we propose structure (III) for the yellow product.

By condensation of 4-biphenylglyoxal with *p*-aminobenzoic acid in aqueous dioxan a product with the formula (I) was obtained. On heating at 140° and 15 mm, this compound eliminated one mole of water and furnished (III). From compound (III), the derivatives (I) or (II) can be obtained by the addition of one mole of water, or one mole of ethanol, respectively. This has been confirmed by the elementary analysis and the infrared spectra of the addition products. (I) and (II) in ethanolic or aqueous medium can be transformed into one another almost quantitatively according to the law of mass action.

In order to clarify further the chemical structure of *p*-[*N*-(α-alkoxy-β-keto-β-*para*biphenyl)ethylamino]benzoic acid (II), and to study the biological absorption of various derivatives of this type, we have prepared a series of condensation products of 4-biphenylglyoxal and *p*-aminobenzoic acid with aliphatic alcohols, saturated and unsaturated, primary and secondary, and with benzyl alcohol and cyclohexanol. In some cases, it was also possible to isolate the intermediate 4-biphenylglyoxal as hemiacetals (Table I).

Experimental

p-(*4-Biphenylglyoxylidene*)*aminobenzoic acid* (*III*). This compound may be obtained in two ways: (*a*) by heating *p*-[*N*-(α-ethoxy-β-keto-β-*para*biphenyl)ethylamino]benzoic acid (II; $R = C_2H_5$) at 140° (14 mm) to constant weight; weight loss, calcd.: 12·26; found: 12·40. The substance melted at 192° (d.).

Anal. Calcd. for $C_{21}H_{15}NO_3$: C, 76·58, H, 4·59; N, 4·25; equiv. wt., 329. Found: C, 77·00; H, 4·65; N, 4·36; equiv. wt., 329.

(*b*) The same compound is formed by heating *p*-[*N*-(α-hydroxy-β-keto-β-*para*biphenyl)ethylamino]benzoic acid (I) as under (*a*). Weight loss, calcd.: 5·18; found: 5·25.

Anal. Found: C, 76·04; H, 4·53; N, 4·34; equiv. wt., 326.

The infrared spectra of the products obtained by methods (*a*) and (*b*) were identical.

p-[N-(α-*Hydroxy-β-keto-β-*para*biphenyl*)*ethylamino*]*benzoic acid* (*I*) was obtained in near-quantitative yield by stirring *p*-(4-biphenylglyoxylidene)aminobenzoic acid (III) in aqueous dioxan at room temperature. The colourless crystals showed m.p. 192° (d.), and the infrared spectrum was identical with that of

the product obtained by direct condensation of 4-biphenylglyoxal with *p*-aminobenzoic acid in aqueous dioxan.

Anal. Calcd. for $C_{21}H_{17}NO_4$: C, 72·61; H, 4·93; N, 4·03; equiv. wt., 347. Found: C, 72·85; H, 5·09; N, 4·01; equiv. wt., 347.

p-[N-(*α-Ethoxy-β-keto-β-*para*biphenyl*)*ethylamino*]*benzoic acid* (*II*). For the preparation of this compound, 4-biphenylglyoxylideneaminobenzoic acid was stirred in ethanol at room temperature, and the colourless crystals thus obtained in nearly quantitative yield were filtered; m.p. 192° (d.).

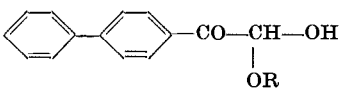
Anal. Calcd. for $C_{23}H_{21}NO_4$: C, 73·58; H, 5·64; N, 3·75; C_2H_5O, 12·04; equiv. wt., 375. Found: C, 72·97; H, 5·68; N, 3·80; C_2H_5O, 11·80; equiv. wt., 374.

The infrared spectrum was identical with that obtained by direct condensation of 4-biphenylglyoxal with *p*-aminobenzoic acid in ethanol.

General Method for the Preparation of 4-Biphenylglyoxal Hemiacetals

Anhydrous yellow 4-biphenylglyoxal was obtained by vacuum distillation of 4-biphenylglyoxal ethyl-hemiacetal, b.p. 145° (0·2 mm). The hemiacetals were prepared by crystallizing this product from the corresponding alcohol (Table I).

Table I. 4-Biphenylglyoxal hemiacetals



R	m.p. °C	% Yield	Empirical formula	Calcd.			Found		
				C	H	OR	C	H	OR
CH_3	95–96	96	$C_{15}H_{14}O_3$	74·36	5·83	12·80	74·45	6·01	12·30
n-C_3H_7	86–87	90	$C_{17}H_{18}O_3$	75·53	6·71	21·86	75·46	6·84	21·20
i-C_3H_7	93–94	85	$C_{17}H_{18}O_3$	75·53	6·71	21·86	75·16	6·84	
n-C_8H_{17}	54–58	85	$C_{22}H_{28}O_3$	76·61	8·29		77·72	8·23	
$CH_2{=}CHCH_2$	93	93	$C_{17}H_{16}O_3$	76·10	6·01		75·80	6·19	
$CH{\equiv}CCH_2$	97	92	$C_{17}H_{14}O_3$	76·67	5·30		76·37	5·45	
$C_6H_5CH_2$	90–92	93	$C_{21}H_{18}O_3$	79·22	5·70		79·02	5·73	

General Method for the Preparation of *p*-*N*[-(α-alkoxy-β-keto-β-*para*biphenyl)ethylamino]benzoic acids

A mixture of 0·01 mole of 4-biphenylglyoxal, 0·01 mole of *p*-aminobenzoic acid, and 40 ml of the corresponding alcohol was stirred at room temperature for 4 h, cooled, and the precipitated crystals were filtered (Table II).

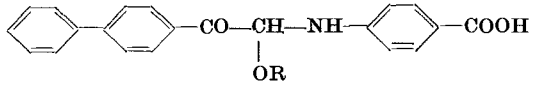
Infrared Absorption Measurements

The infrared spectra were measured in the solid state in KBr pellets ($c = 0 \cdot 25$ per cent) with a Perkin-Elmer spectrophotometer 12C with NaCl optics. The spectra of all the compounds listed in Table III generally resemble the spectrum of compound (II) ($R = C_2H_5$). The variations in the nature of alkyl groups R, however, cause some band displacements. The intensity of the absorption bands of the main functional groups appears to vary according to the concentration of that group in the molecule; e.g., as the weight of the saturated alcoholic residues increases, the CH_2— and CH_3— bands increase in intensity, while that of C—O (ether) band decreases.

Biological data

The screening tests were performed on 8 Swiss strain mice, weighing, on the average, 15–20 g. The drugs were given in aqueous suspension with 3 per cent gum arabic. The suspension for the oral and subcutaneous administration was prepared in such a way that 0·10 ml contained the established dose of the drug. The compounds were administered for 10 days in doses equimolecular to 500 mg of 4-biphenylglyoxal hydrate for the oral administration and to 250 mg of the same substance for the parenteral administration. All the viruses were inoculated 24 h after the beginning of the treatment. The compounds which produced a survival of at least 50 per cent were considered to be significantly active. The PR8 strain of influenza A virus was obtained from the 'Istituto Sieroterapico Italiano' in the form of allantoic fluid of embryonated eggs and was adapted to mice by repeated passage via the nasal route.

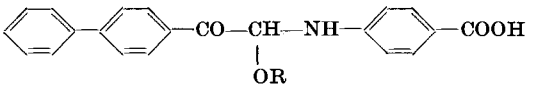
Table II. *p*-[*N*-(α-Alkoxy-β-keto-β-*para*biphenyl)ethylamino]benzoic acids

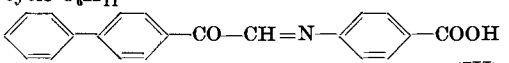


R	m.p. °C	% Yield	Empirical formula	Calcd. C	Calcd. H	Calcd. N	Calcd. OR	Found C	Found H	Found N	Found OR
CH_3[a]	192	83	$C_{22}H_{19}O_4N$	73·11	5·30	3·88	8·57	72·99	5·42	3·85	8·42
n-C_3H_7[a]	192	96	$C_{24}H_{23}O_4N$	74·02	5·95	3·60	15·18	73·98	6·21	3·51	15·00
i-C_3H_7[a]	192	95	$C_{24}H_{23}O_4N$	74·02	5·95	3·60	15·18	73·47	5·38	3·56	15·10
n-C_4H_9	192	98	$C_{25}H_{25}O_4N$	74·42	6·25	3·47		74·15	6·41	3·38	
s-C_4H_9	185–186	87	$C_{25}H_{25}O_4N$	74·42	6·25	3·47		74·17	6·32	3·55	
n-C_5H_{11}	192	93	$C_{26}H_{27}O_4N$	74·80	6·52	3·36		74·54	6·43	3·42	
i-C_5H_{11}	192	89	$C_{26}H_{27}O_4N$	74·80	6·52	3·36		74·84	6·62	3·35	
n-C_8H_{17}	136–138	90	$C_{29}H_{33}O_4N$	75·79	7·24	3·05		76·07	7·33	3·14	
$CH_2=CH—CH_2$[a]	192	95	$C_{24}H_{21}O_4N$	74·40	5·56	3·62		74·36	5·54	3·64	
$CH\equiv C—CH_2$[a]	192	87	$C_{24}H_{19}O_4N$	74·79	4·97	3·63		74·19	5·07	3·84	
$CH_3C(CH_3)=CHCH_2CH_2CH(CH_3)CH_2CH_2$[b]	128–129	70	$C_{31}H_{35}O_4N$	76·67	7·27	2·88		76·78	7·26	3·01	
$C_6H_5CH_2$	184–186	69	$C_{28}H_{23}O_4N$	76·87	5·30	3·20		76·52	5·38	3·20	
cyclo-C_6H_{11}	176–178	85	$C_{27}H_{27}O_4N$	75·50	6·34	3·26		75·33	6·51	3·29	

[a] On heating at 140°/0·2 mm, it was transformed into 4-biphenylglyoxylideneaminobenzoic acid.
[b] Recrystallized from ether-petroleum ether.

Table III. Characteristic infrared absorption frequencies of *p*-[*N*-(α-alkoxy-β-keto-β-*para*biphenyl)ethylamino] benzoic acids



R	ν (N—H)	ν (CH_2)—ν (CH_3)	ν (C=O) con. (COOH) ar.	γ (C—O) ether	γ (C—H) *p*-disubstituted arrangement	γ (C—H) monosubstituted arrangement	ν (C≡H)	ν (CH=CH_2)
H (I)	3408	—	1683	—	844	731–696		
CH_3	3374	2906	1672	1052	845	726–692		
C_2H_5 (II)	3423	2976–2898–2862	1670	1058	849	729–697		
n-C_3H_7	3397	2945–2906–2853	1678	1067–1025[a]	848	729–696		
i-C_3H_7	3385	2954–2890	1678	1031	848	728–696		
n-C_4H_9	3391	2913–2853	1678	1047	845	730–696		
s-C_4H_9	3408	2953–2911–2861	1677	1037	846	728–697		
n-C_5H_{11}	3385	2912–2849	1676	1068–1048[a]	844	728–690		
i-C_5H_{11}	3384	2936–2890–2860	1675	1069	845	728–686		
n-C_8H_{17}	3383	2917–2860	1678	1036	847	729–689		
CH_2=CH—CH_2	3388	2890–2840	1680	1044–1037[a]	848	731–697		990
CH≡C—CH_2	3385	2941–2886–2840	1677	1050	848	729–697	2141	
CH_3—C(—CH_3)=CH—CH_2—CH_2—CH(—CH_3)—CH_2—CH_2	3390	2898–2840	1678	1066	844	728–692		
C_6H_5—CH_2	3389	2850–2845	1675	1058	844	727–695		
cyclo-C_6H_{11}	3397	2924–2849	1675	1044–1028[a]	845	756–697		
 (III)	—	—	1683	—	843	732–695		

[a] The outstanding bands in the absorption zone of the C—O ether group appear to be two. If both are to be attributed to the ether group, their origin may be due to the presence of rotational isomers.

Table IV. Antiviral activities

Drug	Virus A–PR8 nasal inoculation 5 LD50 (LD50 = $10^{-2.13}$) % Survivals Route of Administration		LD50[a] mg/kg
	s.c.	p.o.	
4-$C_6H_5C_6H_4COCHO.H_2O$	42	37	1300
p-[4-$C_6H_5C_6H_4COCH(OC_2H_5)NH]C_6H_4COOH$	71	60	> 1500

[a] Determined in mice weighing 15–20 g. Administration of the compounds in aqueous suspension with 3 per cent gum arabic.

Summary. The structure of *p*-[*N*-(α-ethoxy-β-keto-β-*para*biphenyl) ethylamino]benzoic acid (II) has been established. A series of α-alkoxy analogues of this compound, and of 4-biphenylglyoxal hemiacetals has been prepared. The antiviral activity of (II) has been reported.

Acknowledgements. We wish to thank Prof. M. Reggiani and Dr. B. Casu of the Istituto Ronzoni of Milan for their co-operation in carrying out and interpreting the infrared spectra.

(*Revised manuscript received 9 October, 1959*)

References

[1] Cavallini, G., Massarani, E., Nardi, D., Magrassi, F., Altucci, P., Lorenzutti, G. and Sapio, U. *J. med. pharm. Chem.* **1,** 365 (1959)
[2] Musante, C. and Parrini, V. *Gazz. chim. ital.* **80,** 869 (1950)