The synthesis of some acylglycines and related oxazolones

Article (Published Version)

694. *The Synthesis of Some Acylglycines and Related Oxazolones.*

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A series of acylglycines has been prepared and the ultraviolet absorption spectra of the corresponding oxazolones, obtained with 4-dimethylaminobenzaldehyde and -cinnamaldehyde in the presence of acetic anhydride, have been measured. The sensitivity of these two aldehydes for the detection of the glycines on paper chromatograms has been investigated.

Hippuric acids are usually detected on paper chromatograms by spraying with 4-dimethylaminobenzaldehyde (4%) in acetic anhydride containing sodium acetate (Altman's reagent) and subsequent heating to *ca.* 140° for 1—2 min. The procedure [1] converts the colourless acids into readily visible coloured oxazolones, and it has been stated [2] that the reaction is highly specific for substituted benzoylglycines although it is known to be positive for their vinylogues.[3] About thirty compounds present in extracts of normal human urine give the reaction and although some of these are well recognised an appreciable number remain unidentified.[3] Several of these give very intense colours with Altman's reagent, and it was therefore decided to synthesise a number of new acylglycines, and related oxazolones, to aid further studies of identification.

The glycines listed in Table 3 were prepared by coupling the corresponding acid

[1] Gaffrey, Schreier, Di Ferrante, and Altman, *J. Biol. Chem.*, 1954, **206**, 695.
[2] Smith, "Chromatographic Techniques," Heinemann, 1958, p. 195.
[3] Acheson and Dearnaley, *Canad. J. Biochem. Physiol.*, 1960, **38**, 503; Smith, Paul, McGeer, and McGeer, *ibid.*, 1959, **37**, 1493; Armstrong, Shaw, and Wall, *J. Biol. Chem.*, 1956, **218**, 293.

chlorides, any hydroxyl groups being protected, with either glycine in aqueous alkali or ethyl aminoacetate in ether; the ester and protecting groups, if present, were then hydrolysed. The corresponding 4-*p*-dimethylaminobenzylideneoxazolones (Table 2), free hydroxyl groups being acetylated, were obtained from these glycines, and some others, by treatment with *p*-dimethylaminobenzaldehyde and acetic anhydride. Sodium acetate is often but not always used as a catalyst in similar oxazolone syntheses;[4] it proved unnecessary here, perhaps because of the basic nature of the aldehyde employed. All the glycines gave identical yellow-orange to deep purple colours on paper chromatograms when treated with the aldehyde and acetic anhydride in the presence or absence of sodium acetate.

It has been reported recently[5] that 4-dimethylaminocinnamaldehyde is ten times as sensitive, but less selective, than the usual *p*-dimethylaminobenzaldehyde in the Ehrlich test for pyrroles and indoles. In order to see if a similar replacement would increase the sensitivity of Altman's reagent a number of oxazolones derived from this cinnamaldehyde were prepared (Table 2). Compounds 18 and 19 (Table 1) were very difficult to purify and six crystallisations were necessary before fairly satisfactory analytical results were obtained. The increase in the extinction coefficients of the visible absorption relative to those of the corresponding benzaldehyde derivatives was small although the positions of maximum absorption moved towards the red (Table 1). More disappointing was the

TABLE 1. *Visible and ultraviolet absorption spectra of the oxazolones.*

Compound no.	2-Subst.	4-Subst.*	$\lambda_{max.}$ (mμ) ($10^{-4}\varepsilon$) in neutral EtOAc			$\lambda_{max.}$ (mμ) ($10^{-4}\varepsilon$) in acid EtOAc		
1	Ph	DMB	462 (5·35)	288 (1·1)		360 (3·8)		260 (1·7)
2	*m*-Acetoxyphenyl	DMB	466 (5·3)	303 (1·4)		379 (2·7)	360 (4·1)	260 (1·7)
3	*p*-Acetoxyphenyl	DMB	465 (5·15)	302 (1·1)		362 (3·7)	341 (3·4)	
4	*m*-Methoxyphenyl	DMB	462 (5·5)	301 (1·15)	272 (1·1)	524 (0·4)	361 (3·4)	261 (1·25)
5	*p*-Methoxyphenyl	DMB	459 (4·8)	303 (1·1)		524 (0·4)	372 (3·4)	275 (1·2)
6	3-Acetoxy-4-methoxyphenyl	DMB	460 (5·3)	305 (1·5)		530 (0·2)	371 (4·1)	274 (1·5)
7	3,4-Dimethoxyphenyl	DMB	463 (5·5)	317 (1·1)	302 (1·1)		380 (2·5)	275 (0·9)
8	3,4-Methylenedioxyphenyl	DMB	465 (6·6)	320 (1·2)		379 (4·1)	262 (1·6)	
9	3,4,5-Trimethoxyphenyl	DMB	466 (5·3)	304 (1·3)		537 (0·6)	376 (3·3)	280 (1·3)
10	Styryl	DMB	477 (6·6)	320 (2·1)		550 (0·3)	377 (4·6)	288 (1·6)
11	4-Methoxystyryl	DMB	476 (6·3)	339 (2·2)	260 (1·0)	550 (1·4)	409 (3·4)	308 (1·3)
12	3,4-Dimethoxystyryl	DMB	480 (6·6)	340 (1·6)	262 (1·0)	560 (1·3)	420 (3·2)	
13	3,4-Methylenedioxystyryl	DMB	482 (6·6)	348 (1·7)		435 (3·2)	341 (2·6)	327 (2·5)
14	4-Phenylbuta-1,3-dienyl	DMB	487 (8·3)	340 (4·2)		420 (5·8)	309 (2·5)	
15	Ph	DMC	483 (4·5)	327 (1·4)	260 (1·7)		379 (3·5)	
16	*m*-Methoxyphenyl	DMC	484 (4·6)	325 (1·4)	259 (1·5)		371 (4·5)	274 (0·9)
17	*p*-Methoxyphenyl	DMC	477 (4·1)	334 (1·3)	263 (1·4)		396 (4·4)	290 (0·9)
18	Styryl	DMC	498 (3·4)	344 (1·5)	271 (1·9)		419 (3·1)	292 (1·2)
19	4-Phenylbuta-1,3-dienyl	DMC	505 (7·8)	355 (3·8)		450 (7·8)		

* DMB = *p*-dimethylaminobenzylidene; DMC = 4-dimethylaminocinnamylidene.

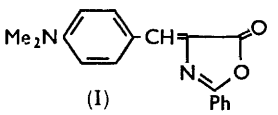
finding that the yellow-brown background colour produced by the 4-dimethylaminocinnamaldehyde (0·2%)–acetic anhydride spray, and subsequent heating at 130—150°, was such that hippuric acid could not be detected after chromatography at concentrations of less than 1·5 μg. cm.$^{-2}$ on Whatman No. 1 paper, while the corresponding figure for the *p*-dimethylaminobenzaldehyde reagent was 0·2 μg. cm.$^{-2}$. A series of attempts to reduce

[4] Carter, "Organic Reactions," 1946, Vol. III, p. 209.
[5] Harley-Mason and Archer, *Biochem. J.*, 1958, **69**, 60P.

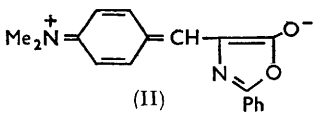
this background, for instance by altering the heating time and temperature, reagent concentration, or by subsequent spraying with acids, was unavailing.

Increasing the conjugation in the substituent at position 4 by one double bond increases the wavelength of maximum absorption of the visible absorption band by 18—22 mμ, and at position 2 by 15—19 mμ, in neutral solution; the changes are not so regular after acidification. Some of the oxazolones showed very shallow broad maxima at *ca.* 550 mμ, but in most cases such maxima could not be distinguished at the concentrations employed. The extinction coefficients for compound 18 (Table 1) are surprisingly low; it is possible that the fair analysis was a misleading representation of the purity of the compound. The visible and ultraviolet absorption spectra of the oxazolones are recorded in Table 1.

As the benzylideneoxazolone ring readily opens in alcoholic solvents yielding cinnamic esters,[6] the spectra (Table 1) were all measured in purified ethyl acetate. The values obtained for 4-*p*-dimethylaminobenzylidene-2-phenyloxazolone (I) are similar to those reported earlier [6] for ether and chloroform solutions. The intense colours of these



(I)



(II)

oxazolones can be related to resonance involving structures such as (II). The addition of concentrated sulphuric acid to the ethyl acetate solutions caused a very marked hypsochromic change in their spectra (Table 1), which can be attributed to the addition of a proton to the dimethylamino-group. This prevents the formation of the charged resonance

TABLE 2. *Oxazolones derived from the appropriate glycine and* p-*dimethylaminobenzaldehyde and -cinnamaldehyde.*

Compound no. (Table 1)	Solvent	Appearance	M. p.	Formula	Found (%) C	Found (%) H	Found (%) N	Required (%) C	Required (%) H	Required (%) N
1	A	Red-brown [c]	210—211° [a]	—	—	—	—	—	—	—
2	B	Pale orange [b]	174	$C_{20}H_{18}O_4N_2$	68·9	5·1	8·3	68·6	5·2	8·0
3	A	Pale orange [b]	180	$C_{20}H_{18}O_4N_2$	68·5	5·3	8·3	68·6	5·2	8·0
4	B, A	Crimson [c]	188	$C_{19}H_{18}O_3N_2$	70·7	5·9	8·8	70·8	5·6	8·7
5	B, A	Orange [c]	191	$C_{19}H_{18}O_3N_2$	70·5	5·6	8·7	70·8	5·6	8·7
6	A, D	Orange [bc]	195	$C_{21}H_{20}O_5N_2$	66·8	5·2	7·1	66·3	5·3	7·4
7	B, A, D	Deep red [d]	219	$C_{20}H_{20}O_4N_2$	68·2	5·7	8·0	68·1	5·7	7·9
8	A, D	Orange [e]	236	$C_{19}H_{16}O_4N_2$	67·8	4·9	8·6	67·8	4·8	8·3
9	B, A	Orange-red [e]	210	$C_{21}H_{22}O_5N_2$	66·1	5·6	—	65·9	5·8	—
10	C, A	Red-purple	203—204	$C_{20}H_{18}O_2N_2$	75·8	6·0	—	75·5	5·7	—
11	B, A	Orange [c]	190	$C_{21}H_{20}O_3N_2$	72·7	5·8	8·2	72·4	5·8	8·0
12	B, A	Orange-red [e]	205	$C_{22}H_{22}O_4N_2$	69·7	5·7	7·7	69·8	5·9	7·4
13	B	Carmine [d]	250—251	$C_{21}H_{18}O_4N_2$	69·7	4·9	8·0	69·6	5·0	7·7
14	E	Dark purple	162	$C_{22}H_{20}O_2N_2$	76·5	5·9	8·2	76·7	5·9	8·1
15	B, A	Purple [c]	198	$C_{20}H_{18}O_2N_2$	75·6	5·6	8·8	75·5	5·7	8·8
16	B, A	Maroon [d]	181—182	$C_{21}H_{20}O_3N_2$	72·6	5·8	8·1	72·4	5·8	8·0
17	B, A	Purple [e]	209	$C_{21}H_{20}O_3N_2$	72·4	5·8	8·0	72·4	5·8	8·0
18	B, A	Deep purple [d]	220	$C_{22}H_{20}O_2N_2$	76·4	5·8	—	76·7	5·9	—
19	B, A	Purple-black	220	$C_{24}H_{22}O_2N_2$	77·0	5·7	—	77·8	6·0	—

A = benzene, B = acetonitrile, C = dioxan, D = nitromethane, E = acetone. [a] Gaffrey *et al.*[1] give m. p. 208—210°; Hellerman, Porter, Lowe, and Koster (*J. Amer. Chem. Soc.*, 1946, **68**, 1890) give 220°. [b] Prepared from the hydroxy-glycines. [c] Needles. [d] Prisms. [e] Plates.

contributor (II). In agreement with this the spectrum of 4-*p*-dimethylaminobenzylidene-2-phenyloxazolone under acid conditions (Table 1) is very similar to that of 4-benzylidene-2-phenyloxazolone ($\lambda_{max.}$ 360 mμ, ε $4{\cdot}2 \times 10^4$, in ether [6]). The possibility that the oxazolone ring is opened by the sulphuric acid has been excluded. The absorption

[6] Bennett and Hoeger, *J. Amer. Chem. Soc.*, 1952, **74**, 5975.

spectrum of 4-*p*-dimethylaminobenzylidene-2-phenyloxazolone in methanol solution changed slowly. At equilibrium the spectrum [$\lambda_{max.}$ 362 mμ ($\varepsilon \times 10^{-4}$, 2·9), 233 mμ (1·85)] was presumably due to the cinnamic ester and was comparable with that of similar esters. Addition of sulphuric acid changed the absorption maxima to 285 (2·0) and 222 mμ (1·6) which are widely different from those of the original oxazolone in acidified ethyl acetate.

γ-Oxo-γ-phenylbutyric acid with *p*-dimethylaminobenzaldehyde and acetic anhydride gave 3-*p*-dimethylaminobenzylidene-2,3-dihydro-5-phenylfuran-2-one which is isosteric with the oxazolone (I). As expected, they have similar absorption spectra in neutral and acid solution, and γ-oxo-γ-phenylbutyric acid on paper chromatograms yielded a yellow-orange spot with Altman's reagent. Although this keto-acid is perhaps an unlikely constituent of urine it is significant that Altman's reagent can give intense colours on paper chromatograms with compounds other than glycine derivatives. Aromatic primary amines give deep yellow colours with the reagent without heating, and these are not intensified with heat. An interesting example is *p*-aminobenzoylglycine which gives a yellow Schiff's base in the cold and subsequent heating yields a yellow-orange oxazolone.

Dr. P. Smith has informed us that our synthetic 3-hydroxy-4-methoxybenzoylglycine (isovanilloylglycine) is chromatographically identical with his specimen obtained from human urine.[7]

Experimental

The absorption spectra were measured by a Carey recording spectrophotometer for *ca.* 10^{-5}M-solutions in alcohol-free ethyl acetate. After acidification with sulphuric acid, so that the acid : oxazolone ratio was *ca.* 10^4 : 1, the spectra were immediately redetermined.

3-*Hydroxy*-4-*methoxybenzoic Acid*.[8]—3-Hydroxy-4-methoxybenzaldehyde (30·4 g.) was added with rapid stirring to freshly prepared silver oxide (from 134 g. of silver nitrate) suspended in 10% aqueous sodium hydroxide (800 ml.). After refluxing (2 hr.) and filtration while hot the filtrate was treated with sulphur dioxide until the pH reached 2·5. 3-Hydroxy-4-methoxybenzoic acid (29·5 g.) separated on cooling, and after one crystallisation from ethanol had m. p. 247° (lit.,[9] m. p. 250°). It was acetylated and subsequently converted into the acid chloride for use in method C.

Acylglycines (*Table* 3).—*Method* A. The acid chloride (0·05 mol.) in ether (24 ml.) was added in 10 min. in three portions to a stirred solution of glycine (0·25 mol.) in aqueous 0·5N-sodium hydroxide (80 ml.). N-Sodium hydroxide (50 ml.; total alkali 0·09 mol.) was added (50 min.) with stirring, so that the pH was kept between 7·4 and 8·0. After filtration the solution was strongly acidified, the acylglycine being precipitated.

Method B. The finely powdered methoxycarbonyloxybenzoyl chloride (3·4 g.) was added to glycine (1·2 g.) in 0·5N-sodium hydroxide (20 ml.) and was followed by more N-alkali (21·5 ml.). After filtration as above, sodium hydroxide (1·9 g.) in water (4·5 ml.) was added and after 15 min. the solution was strongly acidified, then saturated with salt, and the hydroxybenzoylglycine was extracted with ethyl acetate (3 × 100 ml.).

Method C. The acid chloride (0·044 mol.), dissolved or suspended in ether (250 ml.), was added to ethyl aminoacetate[10] (0·099 mol.) in ether (150 ml.). The residue obtained by evaporation of the ether gave on crystallisation the ethyl acylaminoacetate (Table 3). Shaking the ester (0·01 mol.) with sodium hydroxide (0·8 g., 0·02 mol.) in water (5 ml.) for 2 hr. and filtration, followed by acidification, precipitated the free acylglycine; *O*-acetyl and *O*-methoxycarbonyl groups were also hydrolysed.

Oxazolones (*Table* 2).—The hippuric acid (0·005 mol.), *p*-dimethylamino-benzaldehyde or -cinnamaldehyde (0·005 mol.), and acetic anhydride (3 ml.) were refluxed (20 min.) and poured into water. After ½ hr. the product was filtered off, dried, and crystallised.

3-p-*Dimethylaminobenzylidene*-2,3-*dihydro*-5-*phenylfuran*-2-*one*.—γ-Oxo-γ-phenylbutyric acid

[7] Hill, Ratcliffe, and Smith, *Chem. and Ind.*, 1959, 399.
[8] Cf. Pearl, *J. Amer. Chem. Soc.*, 1946, **68**, 429.
[9] Späth and Burger, *Ber.*, 1926, **59**, 1494; Fischer and Freudenberg, *Annalen*, 1911, **384**, 237.
[10] Fischer, *Ber.*, 1901, **34**, 436.

(1·05 g.) was refluxed for 10 min. with acetic anhydride (12 ml.), after which *p*-dimethylaminobenzaldehyde (0·9 g.) was added and refluxing continued for 30 min. The solid (0·58 g.), which separated on cooling to 0°, gave on successive crystallisation from benzene, acetonitrile, and benzene the *furanone* as crimson plates, m. p. 163—164° (Found: C, 78·2; H, 5·9; N, 4·8.

TABLE 3. *Acylglycines and their ethyl esters.*

Acylglycine	Method	Solvent	Appearance	Yield (%)	M. p.	Formula	Found (%) C	H	N	Required (%) C	H	N
m-Hydroxybenzoyl [a]	B	W	Prisms	42	185°	—	—	—	—	—	—	—
p-Hydroxybenzoyl	B [b]	W	Prisms	41	234	$C_9H_9O_4N$	55·5	4·6	7·4	55·4	5·0	7·2
m-Methoxybenzoyl	A	W–E	Prisms	88	120 [c]	$C_{10}H_{11}O_4N$	57·4	5·3	6·7	57·4	5·4	6·9
3,4-Dimethoxybenzoyl	A	W–E, E	Plates	40	190 [d]	$C_{11}H_{13}O_5N$	55·0	5·5	5·7	55·2	5·5	5·9
3,4-Methylenedioxybenzoyl	C	W	Needles	64	178 [e]	—	—	—	—	—	—	—
3-Hydroxy-4-methoxybenzoyl	C	W	Prisms	63	203·5	$C_{10}H_{11}O_5N$	53·0	4·8	6·4	53·3	4·9	6·2
3,4,5-Trimethoxybenzoyl	C	W–E	Needles	76	215 [f]	$C_{12}H_{15}O_6N$	53·5	5·5	—	53·5	5·6	—
4-Methoxycinnamoyl	A	E	Needles	47	169	$C_{12}H_{13}O_4N$	61·3	5·6	6·0	61·4	5·7	5·7
3,4-Dimethoxycinnamoyl	A	W, E	Needles	90	182	$C_{13}H_{15}O_5N$	58·9	5·7	—	58·8	5·5	—
3,4-Methylenedioxycinnamoyl	C	W, W–E	Needles	80	209	$C_{12}H_{11}O_5N$	57·7	4·6	5·7	57·8	4·4	5·6
5-Phenylpenta-2,4-dienyl	A	W–E	Needles	76	216	$C_{13}H_{13}O_3N$	67·9	5·8	—	67·6	5·7	—
Ethyl acylaminoacetates												
3-Acetoxy-4-methoxybenzoyl	C	W–E	Plates	93	137	$C_{14}H_{17}O_6N$	56·9	5·7	4·7	56·9	5·8	4·7
3,4-Methylenedioxybenzoyl	C	W	Needles	78	88 [g]	—	—	—	—	—	—	—
3,4,5-Trimethoxybenzoyl	C	W–E	Needles	75	109 [h]	$C_{14}H_{19}O_6N$	56·8	6·4	4·9	56·5	6·4	4·7
3,4-Methylenedioxycinnamoyl	C	W, E–W	Needles	26	135·5	$C_{14}H_{15}O_5N$	60·6	5·5	5·1	60·7	5·4	5·1
3,4-Dimethoxycinnamoyl	C	E–W	Rhombs	95	108	—	—	—	—	—	—	—

Solvents: E = ethanol; W = water. [a] Previously prepared by the hydrolysis of ethyl 3-acetoxybenzoylaminoacetate (Armstrong, Wall, and Porter, *J. Biol. Chem.*, 1956, **218**, 921) and by the diazotisation of 3-aminohippuric acid (Griess, *Ber.*, 1868, **1**, 190; Conrad, *J. prakt. Chem.*, 1877, **15**, 259. [b] Fischer (*Ber.*, 1908, **41**, 2880) briefly describes this synthesis and gives m. p. 238° (decomp.); Bray, Ryman, and Thorpe (*Biochem. J.*, 1947, **41**, 212) give m. p. 239—240°. [c] Quick (*J. Biol. Chem.*, 1932, **97**, 403) gives m. p. 122° for material isolated from human urine. [d] Kametani and Iwakata (*J. Pharm. Soc. Japan*, 1950, **70**, 258) give m. p. 191°. [e] *Idem* (*ibid.*, p. 263) give m. p. 178° from a synthesis by method A. [f] *Idem* (*ibid.*, p. 258) give m. p. 218°. [g] Sugasawa (*ibid.*, 1935, **55**, 224) give m. p. 109° from another synthesis. [h] Kametani *et al.* (*ibid.*, p. 263) give m. p. 89° from another synthesis.

$C_{19}H_{17}NO_2$ requires C, 78·3; H, 5·9; N, 4·8%). Crystallisation from benzene above *ca.* 40° gave a deep purple modification, which became red at *ca.* 150°, and had m. p. and mixed m. p. with the crimson form, 163—164°. The purple form reverted to the crimson form when seeded in benzene suspension. In ethyl acetate the furanone showed $\lambda_{max.}$ 436 mμ ($10^{-4}\varepsilon$ 5·0), 303 (0·8), 241 (2·2), and after acidification 388 (3·0), 255 (2·1).

We thank Mr. A. O. Plunkett for the spectral measurements and for general technical assistance. Gifts of chemicals from Dr. E. Schlittler of Ciba Pharmaceuticals Inc., Dr. D. A. Peak of Boots Pure Drug Company Ltd., and Dr. J. B. Jepson are gratefully acknowledged. This work was supported by a grant from the Rockefeller Foundation to this Department.

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[*Received, March 15th,* 1960.]