Promotion of Carbohydrate Oxidation in the Heart by Some Phenylglyoxylic Acids

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A series of phenylglyoxylic acids is described, many of which are able to promote carbohydrate oxidation in muscle tissue, thereby favorably altering the carbohydrate/fatty acid balance in situations where fatty acid utilization is elevated. Such situations are reported to occur in ischemic heart disease, particularly following myocardial infarction. In an attempt to effectively deliver the phenylglyoxylic acids to the site of action within the cell, the L-(+)-phenylglycines were employed as prodrugs. These are known to be transaminated to phenylglyoxylic acids. L-(+)-2-(4-Hydroxyphenyl)glycine (**25**, oxfenicine) has been selected for clinical evaluation.

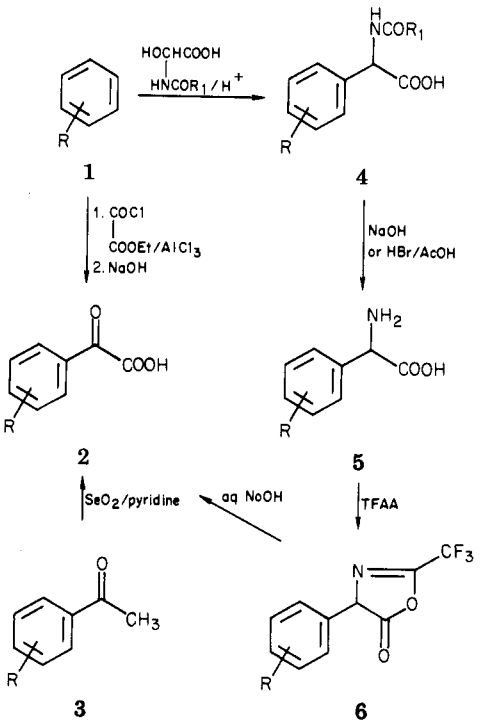
The heart is an organ which derives a high proportion of its energy production from the oxidation of fatty acids in preference to glucose.[1] In ischemic heart disease, and particularly following myocardial infarction, the shift to free fatty acid utilization may be further accentuated by the elevation of plasma levels of catecholamines and free fatty acids.[2] Elevated free fatty acid levels are detrimental to the ischemic heart, since such a situation increases the size of an experimental infarct,[3] increases O_2 consumption without altering contractile performance,[4] and increases the incidence of postinfarction dysrhythmia.[5]

On the other hand, lowering free fatty acids with β-pyridylcarbinol[3] or treatment with dichloroacetic acid[6] reduces the degree of epicardial ST-segment elevation following coronary occlusion in the dog. In anginal patients, antilipolytic therapy produces a significant reduction of exercise-induced ST-segment depression.[7] Stimulation of glucose utilization by the infusion of glucose–insulin–potassium reduces the extent of myocardial necrosis following coronary occlusion in the dog,[8] while in patients with coronary artery disease such treatment reduces myocardial oxygen requirements by decreasing free fatty acid utilization.[9]

Current therapy of the ischemic myocardium is aimed at either reducing work load on the heart (nitrites and vasodilators) or directly reducing heart rate and contractile performance (β-adrenoreceptor blocking drugs). An attractive alternative would be to seek agents which increased the efficiency of myocardial energy production and enhanced the ratio of carbohydrate to fatty acid utilization. Such agents may also reduce the production of lactic acid, the accumulation of which may contribute to the irreversible cell damage observed in ischemic areas of the myocardium.

We describe a series of phenylglyoxylic acids (and related phenylglycines) which are able to promote carbohydrate oxidation when this is depressed by high levels of fatty acid utilization.

Scheme I[a]



[a] R_1 = Ph or OCH_2Ph.

Chemistry. The glyoxylic acids (**2**) were prepared by standard methods, as shown in Scheme I. The nature and desired position of the substituent R, coupled with the availability of suitable starting materials, determined the route that was to be employed. Thus, Friedel–Crafts acylation[10] with ethyloxalyl chloride on **1** gave the phenylglyoxylic ester from which the acid **2** was obtained by alkaline hydrolysis. Alternatively, substituted acetophenones **3** were oxidized with selenium dioxide[11,12] in pyridine to afford **2** directly. Occasionally, the availability of certain phenylglycines, **5**, led to the synthesis of **2** via the oxazolone[13] **6**. This route became the method of choice for the synthesis of 4-hydroxyphenylglyoxylic acid (**11**).

4-(Hydroxymethyl)phenylglyoxylic acid (**14**) was prepared from the ester of 4-tolylglyoxylic acid (**8**). Treatment of this with *N*-bromosuccinimide in carbon tetrachloride solution gave the bromomethyl compound **34**, which was

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reacted with silver acetate according to the method of Finch and Schlittler[14] to give the acetoxymethyl compound **35**. Hydrolysis with excess aqueous NaOH then afforded **14**.

Although the DL-phenylglycines **5** could be obtained from the appropriate benzaldehyde via the Strecker synthesis, it was found more convenient to use the amidoalkylation method as reported by Ben-Ishai, Satati, and Berler.[15] Thus, α-hydroxyhippuric acid was reacted with **1** in a sulfuric acid/acetic acid mixture to afford para-substituted *N*-benzoylphenylglycine (**4**), from which **5** was obtained by hydrolysis. Conversion of the racemic phenylglycines into L-(+) and D-(−) isomers was achieved by N-chloroacetylation and selective hydrolysis of the L-(+)-*N*-(chloroacetyl) isomer with hog kidney acylase enzyme (acylase 1) to give the L-(+)-phenylglycine. The D-(−) isomer was obtained by acid hydrolysis of the recovered D-(−)-*N*-(chloroacetyl) isomer.

Biology. One of the principal mechanisms by which the inhibition of carbohydrate utilization in the presence of an excessive free fatty acid supply is achieved is by preventing the entry of pyruvate into oxidative pathways[16] through the inhibition of the multienzyme complex pyruvate dehydrogenase (PDH).[17,18] Associated with this complex are two regulatory enzymes: PDH-kinase, which converts PDH from its active form (PDHa) to its inactive form (PDHi) by phosphorylation of the pyruvate decarboxylase unit, and PDH-phosphate phosphatase, which catalyzes the reverse reaction and thus reactivates the system.[17-19] An increase in the rate of phosphorylation by the deactivating enzyme PDH-kinase is induced by an increase in the acetyl-CoA/CoA and NADH/NAD ratios.[20,21] A high level of lipid utilization causes an increase in these ratios, and pyruvate utilization is depressed accordingly.[21] In the rat heart, for example, PDH activity was found to be inversely related to serum free fatty acid levels,[16] while in the dog, elevation of plasma free fatty acid levels increased the extraction of free fatty acids by the heart at the expense of glucose, lactate, and pyruvate.[22]

Dichloroacetic acid (DCA) stimulates pyruvate oxidation by inhibiting PDH-kinase[23-25] and thereby raising the proportion of PDH in its active form. This reverses the depressive effects of fatty acids on glucose, lactate, and pyruvate metabolism.[22] Our interest in PDH arose in the context of ischemic heart disease, since its stimulation, either directly or indirectly, appeared to offer a means of switching myocardial metabolism away from fatty acid utilization to that of glucose, which is more economical in terms of oxygen consumption.[1]

Thus, in order to identify compounds able to promote carbohydrate oxidation, a screening system involving the PDH enzyme complex was employed. Throughout this work DCA was used as the reference compound.

To depress the levels of PDHa in the tissues under study, male rats were fed on a high-fat diet (60–70% of calories as fat) for at least 10 days. Two tissues were studied, diaphragm and heart, the former being convenient for primary in vitro evaluation. For this evaluation the diaphragms were removed from the animals and divided into four. Each piece was randomly allocated to one of the control or treatment groups. The pieces were preincubated in Krebs–bicarbonate solution (pH 7.4) containing various concentrations of drug. [^{14}C]Pyruvate was then added, and incubation was continued for 1 h. The amount of $^{14}CO_2$ produced was then measured and the percentage increase over the control noted.

The PDH activity in vivo of the rat heart was measured following subcutaneous administration of the compound to be studied to fat-fed rats. At fixed times after dosing, the rats were killed, their hearts rapidly transferred into liquid nitrogen and pulverized, and the resulting tissue powder was homogenized in an ice-cold extraction medium. A portion of this extract was assayed directly for PDHa. A further portion was incubated in the presence of Mg^{2+} and Ca^{2+} to fully activate the enzyme, which was then assayed for total PDH activity. The PDH activity of both extracts was determined by the decarboxylation of [^{14}C]-pyruvate to produce $^{14}CO_2$. The control experiments gave PDHa levels in fat-fed rat hearts of about 10% of PDH total. This was in accord with published data.[23]

Results and Discussion

In seeking agents able to promote carbohydrate oxidation attention was directed toward α-keto acids following our observation that phenylglyoxylic acid (**7**; Table I) increased the level of PDHa in the heart. This was a particularly interesting finding, since pyruvic acid and, to a smaller degree, 2-oxobutyric acid and phenylpyruvic acid were known to protect PDH from inactivation by the kinase.[26] Furthermore, it had been suggested that PDH contained a regulatory site which was involved in the inactivation of the enzyme by phosphorylation and which bound protective molecules such as pyruvate or phenyl pyruvate.[26,27] However, while this initial rationale for the activity of **7** proved useful, later detailed studies showed that interaction with the regulatory site was not involved. The level of activity shown by **7** was weaker than that of the reference compound DCA; nevertheless, **7** represented a promising base on which to mount a synthetic program. Accordingly, a range of phenylglyoxylic acids bearing various substitutents on the phenyl ring was prepared and tested (Table I). Activity, as compared with **7**, was increased with compounds **9**, **11**, **16**, **17**, and **18**, taking into account individual DCA standard values as a reference point. All these compounds, with the exception of the 4-hydroxy analogue **11**, gave good activity in vivo, after sc administration, although only the 4-(ethylthio) compound **17** approached the level of DCA. Compounds **12–15** were totally devoid of activity, as was the 2-methoxy analogue **20**. Attempts to improve the activity of **11** by additional substitution in the 3 and 3,5 positions of the phenyl ring (**21–23**) were unsuccessful.

Since the activity of a drug is dependent on its concentration at the site of action, we attempted to increase the in vivo activity and prolong the duration of action of

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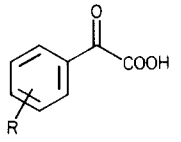
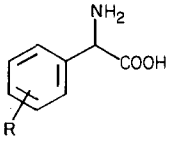
Table I



7–23



24–32

no.	R	mp °C	recrystn solvent	formula[d]	% yield	% stimulation of in vitro rat diaphragm[b] [^{14}C]pyruvate → $^{14}CO_2$: compd 5 mM	compd 2 mM	DCA 0.5 mM	DCA 2 mM	in vivo rat heart sc admn:[d] % PDH active/PDH total: compd, 1.2 mmol/kg	DCA,[c] 1.2 mmol/kg
7	H	66[e]		$C_8H_6O_3$			85 ± 17[z]	42 ± 22[z]	219 ± 49[z]	38 ± 2[z]	87 ± 6[z]
8	4-CH_3	96–98[f]	toluene/hexane	$C_9H_8O_3$	62		43 ± 4	50 ± 30	100 ± 22	20 ± 6	97 ± 9
9	4-OCH_3	90–91[g]	toluene/petr ether	$C_9H_8O_4$	20	202 ± 24	132 ± 26	62 ± 17	161 ± 30	49 ± 1	97 ± 9
10	4-Cl	88–91[h]	toluene	$C_8H_5ClO_3$	15	71 ± 24	48 ± 23		82 ± 20		
11	4-OH	174–176[i]	Et_2O/petr ether	$C_8H_6O_4$	70	106 ± 30	80 ± 11	63 ± 23	142 ± 33	12 ± 1	96 ± 2
12	4-NMe_2	194–195[j]	H_2O/MeOH	$C_{10}H_{11}NO_3$	68	16 ± 14	−5 ± 6	1 ± 9	156 ± 40		
13	4-$CONH_2$	216–220	EtOAc/hexane	$C_9H_7NO_4$[x]	64	20 ± 7	11 ± 11		200 ± 46		
14	4-CH_2OH	119–120	EtOAc/hexane	$C_9H_8O_4$	49		23 ± 7	12 ± 5	108 ± 10		
15	4-$EtSO_2NH$	156–158	EtOAc/hexane	$C_{10}H_{11}NO_5S$	47		22 ± 9	16 ± 7	100 ± 22		
16	4-SCH_3	111–113[k]	toluene	$C_9H_8O_3S$	51	154 ± 17	123 ± 16		101 ± 21	69 ± 6	82 ± 5
17	4-SC_2H_5	41–45[l]	toluene	$C_{10}H_{10}O_3S$[y]	32		231 ± 11	164 ± 43	158 ± 23	73 ± 9	82 ± 5
18	4-S-*i*-C_3H_7	47–50	H_2O/EtOH	$C_{11}H_{12}O_3S$	17		162 ± 35	26 ± 14	200 ± 46	51 ± 19	96 ± 2
19	4-S-*n*-C_4H_9	36–37	toluene/petr ether	$C_{12}H_{14}O_3S$	35		113 ± 44	76 ± 10	200 ± 46	14 ± 3	96 ± 2
20	2-OCH_3	99–102[m]	Et_2O/petr ether	$C_9H_8O_4$	33	−2 ± 7	−10 ± 6		163 ± 56		
21	4-OH; 3-CH_3	167–169	Et_2O/petr ether	$C_9H_8O_4$	55		21 ± 16	2 ± 20	107 ± 9	31 ± 7	89 ± 8
22	4-OH; 3-$CH_2CH{=}CH_2$	126–127	Et_2O/petr ether	$C_{11}H_{10}O_4$	52		29 ± 26	11 ± 2	120 ± 15	11 ± 3	90 ± 7
23	4-OH; 3,5-I_2	162–163[n]	H_2O	$C_8H_4I_2O_4$	9		85 ± 7	5 ± 6	120 ± 15	12 ± 6	87 ± 9
L-24	H	257–261[p]		$C_8H_9NO_2$		10 ± 4			90 ± 8	13 ± 2	87 ± 6
L-25	4-OH	230 d[q]	H_2O	$C_8H_9NO_3$	60	172 ± 26	136 ± 23	84 ± 9	123 ± 10	82 ± 6	87 ± 6
D-26	4-OH	219–220[r]	H_2O	$C_8H_9NO_3$	46	−6 ± 2	26 ± 10		130 ± 40	26 ± 7	87 ± 6
L-27	4-OCH_3	218 d[s]	H_2O	$C_9H_{11}NO_3$	43	115 ± 19	124 ± 18	79 ± 8	122 ± 20	5 ± 2	90 ± 8
D-28	4-OCH_3	249–250[t]	H_2O	$C_9H_{11}NO_3$	67	−14 ± 22	17 ± 7		108 ± 10	11 ± 6	87 ± 9
DL-29	4-SCH_3	241–242[u]	H_2O/EtOH	$C_9H_{11}NO_2S$	41	28 ± 36	65 ± 24		132 ± 23	20 ± 7	76 ± 8
DL-30	4-SC_2H_5	237 d	H_2O/EtOH	$C_{10}H_{13}NO_2$	25	24 ± 7	20 ± 12		132 ± 20	5 ± 10	80 ± 37
L-31	4-Cl	206 d[v]	H_2O/EtOH	$C_8H_8NO_2$	23		102 ± 2	43 ± 6	99 ± 38	43 ± 4	90 ± 7.5
L-32	4-CH_3	216–218[w]	H_2O/EtOH	$C_9H_{11}NO_2$	28		36 ± 2	24 ± 25	95 ± 23		
control									17 ± 4		17 ± 4

[a] All novel compounds were analyzed for C, H, and N. [b] Percent conversion of [^{14}C]pyruvate to $^{14}CO_2$: results given are the mean of four to six determinations at each concentration. [c] DCA = dichloroacetic acid. [d] Results given are the mean of three to six determinations. [e] Sample purchased from the Aldrich Chemical Co. [f] Lit.[32] mp. 98–99 °C. [g] Lit.[10] mp 93 °C. [h] Lit.[33] mp 94–95 °C. [i] Lit.[10] mp 177.5–178 °C. [j] Lit.[34] mp 186–187 °C. [k] Lit.[35] mp 110–111 °C. [l] Contains 0.75 volume water of recrystallization; lit.[22] mp 72–73 °C. [m] Lit.[36] mp. 102–103 °C. [n] Lit.[37] mp 164–166 °C. [p] Lit.[38] $[\alpha]^{26}_D$ +114° (H_2O). [q] $[\alpha]^{26}_D$ +124.5°; lit.[39] mp 225 °C. [r] $[\alpha]^{26}_D$ −148°; lit.[39] mp 225 °C. [s] $[\alpha]^{20}_D$ +137°; lit.[40] $[\alpha]^{25}_D$ +150.4°. [t] $[\alpha]^{25}_D$ −140°; lit.[40] $[\alpha]$ −149.9°. [u] Lit.[41] mp 230–231 °C. [v] $[\alpha]^{20}_D$ +133°. [w] $[\alpha]^{25}_D$ +152.3°. [x] Hemihydrate. [y] Contains 0.75 mol of H_2O. [z] Standard error.

these phenyl glyoxylates by delivering them into the cells as a prodrug. Amino acids are known to be efficiently transported through lipid membranes by means of an active-transport process.[28] In addition, it is known that L-(+)-α-amino acids are converted to α-keto acids by transaminase enzymes.[29]

The L-(+)-phenylglycines **24**, **25**, **27**, **31**, and **32** corresponding to the glyoxylates **7**, **11**, **9**, **10**, and **8** were therefore prepared and tested. The 4-chloro analogue **31** showed moderate in vitro and in vivo activity, but L-(+)-phenylglycine **24** and the 4-methyl analogue **32** were essentially inactive. However, the 4-hydroxy and 4-methoxy analogues **25** and **27** showed in vitro activity at least as good as the reference compound DCA. Surprisingly, **27** was inactive in vivo; **25**, however, produced an 82% stimulation of PDH activity when administered to rats at the standard dose of 1.2 mmol/kg sc. The sc ED_{50} values for **25** and DCA in the rat were 0.3 and 0.4 mmol/kg, respectively.

The DL-(alkylthio)glycines **29** and **30** were essentially inactive both in vitro and in vivo. This was disappointing in view of the good intrinsic activity possessed by the corresponding keto acids **16** and **17**. Subsequent studies in dogs with glycine **29** showed that both the plasma concentration and urinary excretion of the keto acid **16** were very low following po administration of **29**. These low keto acid levels may be due to poor absorption of the glycine from the gastrointestinal tract or else a result of a low degree of transamination.

Compound **25** was selected for further evaluation because it was effective for over 3 h in stimulating rat heart PDH activity whether given by po, iv, or sc routes of administration.[30,31]

Transaminase inhibition studies in vitro (diaphragm tissue) using aminooxyacetic acid at 2 mM as inhibitor showed that pyruvate oxidation was appreciably inhibited by **25** at 2 nM and at 1 mM was completely inhibited.[31] Pharmacokinetic studies in rat, dog, and man have shown that the keto acid **11** appears in the blood soon after administration of **25**. These results suggest that **25** is functioning as a prodrug for 4-hydroxyphenylglyoxylic acid (**11**).

Furthermore, at physiological pH, 4-hydroxyphenylglyoxylic acid (**11**) exists as a hydrophilic dianion. Such compounds pass through lipid membranes only with difficulty. Therefore, when the phenylglycine **25** is transaminated in the cell, the intracellular levels of **11** must build up sufficiently for the compound to exert the desired biological effect.

The D-(−)-amino acids **26** and **28** did not affect pyruvate oxidation either in vitro or in vivo, presumably because these compounds were not transaminated. In man the maximum plasma levels of **11** occurred 2–3 h after oral dosing with **25**, the half-life of excretion of **11** being 4.5 h. Measurement of urine concentrations of **11** indicated a 52–94% recovery over 24 h.

Neither **25** (oxfenicine) nor **11** (UK-22486) had any effect on the pyruvate dehydrogenase-kinase activity in rat heart homogenates, neither did they affect the phosphatase.[31] These compounds differ, therefore, in their mode of action from DCA.

Compound **25** is currently undergoing clinical trials in ischemic heart disease.

Experimental Section

Biology. A. Pyruvate Oxidation in Diaphragms.[31] Male rats (Sprague–Dawley; weight approximately 125 g) were fed on a high fat diet for at least 10 days (the diet consisted of 27% casein, 3% starch, 40% avicel, 2% vitamin supplement, 3% salts mix, and 25% beef dripping by weight). The rats were killed by stunning and cervical dislocation, and the diaphragms were excised into Krebs–Ringer bicarbonate (KRB) buffer, pH 7.4, at 37 °C. Each diaphragm was divided into four portions, which were weighed and placed in flasks containing KRB (3.8 mL). The flasks were gassed with O_2/CO_2 (18:1), stoppered, and incubated at 37 °C in a shaker bath for 1 h. One portion of the diaphragm was incubated with KRB alone (control), the other three portions being incubated with test/standard compound. After 1 h, 0.2 mL of 1- or 2-^{14}C labeled sodium pyruvate was introduced into the flask. The final concentration of sodium pyruvate was 4 mM (specific activity 6.25 nCi/mol). After this addition, the flask was regassed, restoppered, and incubated at 37 °C for 1 h. At the end of this time, a glass center well containing a gelatin capsule with a glass fiber wick and 1 M hyamine hydroxide (0.5 mL) in MeOH were introduced into the flask. The stopper was replaced and 1 mL of 2.5 M H_2SO_4 was injected into the flask, thus terminating the reaction and releasing dissolved CO_2 into the gaseous phase from where it was absorbed by the hyamine hydroxide. After 30 min, the gelatin capsule and hyamine hydroxide were transferred to a scintillation vial containing a 9:1 toluene/ethanol scintillation fluid (8 mL) and assayed for ^{14}C by liquid scintillation counting. Blanks were included, in the absence of a diaphragm portion, to assess the degree of spontaneous decarboxylation of pyruvate. Pyruvate decarboxylation was measured as the amount of [1-^{14}C]pyruvate converted to $^{14}CO_2$ (μmol/g of wet weight of diaphragm tissue), and the results were expressed as percent stimulations per inhibitions with reference to the control incubations.

B. Pyruvate Dehydrogenase Activity in Vivo.[31] Male rats (weight ~125 g) were fed on a high-fat diet for 21 days as described above. One and a half hours after administration of the test compound/placebo, the animals were sacrificed by stunning and cervical dislocation. The heart was rapidly excised, blotted, and frozen in liquid N_2 until use, and then it was powdered in a precussion mortar and cooled in liquid N_2 and a weighed portion of the powder was homogenized in an ice-cold extraction medium (10 mL/g) with a Plytron PT10 tissue homogenizer. The extraction medium contained potassium phosphate buffer (11 mM), pH 7.4, 2.5 mM EDTA, 1.2 mM dithiothreitol, 9 mM sodium pyruvate. A portion of the extract was assayed for pyruvate dehydrogenase (PDH active). A further portion was incubated at 30 °C for 20 min with 5 mM $MgCl_2$ and 2.5 mM $CaCl_2$ to fully activate the enzyme, which was then assayed (PDH total). Extracts were stable on ice for at least 1 h. To assay the activity of PDH active and PDH total, 0.1 mL of each extract was injected into a sealed conical flask containing 0.9 mL of assay medium and incubated at 30 °C. The assay medium consisted of 11 mM potassium phosphate buffer, pH 7.4, 1.2 mM dithiothreitol, 2.8 mM $MgCl_2$, 0.16 mM coenzyme A, 1.6 mM NAD, 0.08 mM thiamine pyrophosphate, 0.1 mM sodium [1-^{14}C]pyruvate (specific activity 0.4 μCi/μmol). PDH releases the 1-^{14}C atom of the pyruvate as $^{14}CO_2$, and PDH was thus assayed by the rate of production of $^{14}CO_2$ as described in A above. Results were calculated in units per gram of wet weight and were finally expressed as percent PDH active per PDH total. At least three rats per group are required to obtain satisfactory results.

Chemistry. All melting points are uncorrected and were obtained using an Electrothermal capillary melting point apparatus. The structures of all compounds were confirmed by their IR and NMR spectra, the latter being determined in either $CDCl_3$ or Me_2SO-d_6. The IR spectra were obtained with a Perkin-Elmer 237 spectrophotometer and the NMR spectra with a Varian

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Associates spectrometer, Model A-6A. The optical rotations were carried out on a Perkin-Elmer 141 spectrometer using a 1% solution in 1 N HCl.

4-(Methylthio)phenylglyoxylic Acid (16). Aluminum chloride (10 g, 0.075 mol) was added portionwise with stirring and cooling to thioanisole (9 g, 0.072 mol) and ethoxalyl chloride (10 g, 0.073 mol) in dry dichloroethane (100 mL). The mixture was stirred for 1 h with cooling, allowed to warm up to room temperature, and stirred for a further 2 h. The mixture was then added to ice/concentrated HCl, and the aqueous and organic layers were separated. The aqueous layer was extracted with $CHCl_3$ (3 × 100 mL), and the organic extracts were combined, washed with H_2O (2 × 100 mL), dried, and evaporated to yield the ester as a green oil, 4.7 g (29%).

The crude ester (4.7 g) was added to a solution of NaOH (3 g) in H_2O (50 mL), and the mixture was heated on a steam bath for 1.5 h before cooling and washing with Et_2O (2 × 25 mL). The aqueous layer was then acidified and extracted with Et_2O (3 × 50 mL), and the ether extract was washed, dried, and evaporated to yield the acid **16**, which was recrystallized from toluene to give **16**: yield 2.1 g (51%); mp 111–113 °C (lit.[35] mp 110–111 °C).

Compounds **8** and **9** were also prepared by this method. Compound **12** was prepared by a known procedure[34] using oxalyl chloride.

4-(Ethylthio)phenylglyoxylic Acid (17). 4-(Ethylthio)-acetophenone (9 g, 0.05 mol) was added to selenium dioxide (6.1 g, 0.061 mol) in dry pyridine (75 mL), and the mixture was heated overnight on a steam bath. Precipitated selenium was filtered off, and the filtrate was evaporated to remove the pyridine. The residual dark oil was partitioned between 2 N HCl and Et_2O, and the layers were separated. The aqueous layer was extracted with Et_2O (3 × 50 mL), and the combined ether extracts were extracted with aqueous $NaHCO_3$ solution (100 mL). The latter was washed with Et_2O and then acidified to give a yellow oil, which was again extracted into Et_2O; the ethereal solution was then washed, dried, and evaporated. Trituration of the resulting oil with 40–60 °C petroleum ether afforded **17** as a solid, which was recrystallized from toluene to give **17**: yield 3.4 g (32%); mp 41–45 °C (lit.[12] mp 72–73 °C). Analytical data shows that **17** contains $0.75H_2O$ water of crystallization.

Compounds **10**, **15**, **18**, **19**, and **20** were prepared by this method.

4-Carbamoylphenylglyoxylic Acid (13). 4-Cyanoacetophenone was oxidized with selenium dioxide in pyridine as described above to give 4-cyanophenylglyoxylic acid (**33**), mp 121–123 °C from toluene. Anal. ($C_9H_5NO_3$) C, H, N. **33** (1.3 g) was then added portionwise with stirring and cooling to cooncentrated H_2SO_4 (6 mL), and the resulting mixture was stirred for 0.75 h; it was then cautiously added to ice–water, and the precipitate was collected, washed with H_2O, triturated with ether, recollected, and dried. Recrystallization from EtOAc/hexane gave pure **13** as the hemihydrate: yield 0.9 g (64%); mp 216–220 °C. Anal. ($C_9H_7NO_4 \cdot 0.5H_2O$) C, H, N.

Ethyl 4-(Bromomethyl)phenylglyoxylate (34). The ethyl ester of 4-toluylglyoxylic acid (**8**; 77.0 g, 0.401 mL) was dissolved in carbon tetrachloride (250 mL) and the solution was heated at reflux temperature. To this was added a mixture of *N*-bromosuccinimide (77.0 g, 0.433 mol) and benzoyl peroxide (0.6 g), and the heating was continued for 3 h. Upon cooling the mixture was filtered, and the filtrate was washed with H_2O, dried, and evaporated to give an oil that solidified on standing. Recrystallization from EtOAc/hexane gave 55 g (51%) of **34**, mp 56–58 °C. Anal. ($C_{11}H_{11}BrO_3$) C, H.

Ethyl 4-(Acetoxymethyl)phenylglyoxylate (35). Ethyl 4-(bromomethyl)phenylglyoxylate. (**34**; 8.0 g, 0.03 mol) was heated under reflux with silver acetate (7.4 g, 0.044 mol) in glacial acetic acid (22 mL) for a total of 0.75 h. The mixture was then cooled and poured into ice–water (400 mL) containing $NaHCO_3$ (40 g). After the frothing had subsided, Et_2O (100 mL) was added to the weakly basic solution, and the mixture was shaken and then filtered. Et_2O (100 mL) was added to the filtrate and the organic layer separated. After further extraction of the aqueous layer with Et_2O (100 mL), the combined organic extracts were washed, dried, and evaporated to give a mobile liquid. Distillation under reduced pressure afforded pure **35**: yield 5.4 g (73%); bp 198–200 °C at (10 mm). Anal. ($C_{13}H_{14}O_5$) C, H.

4-(Hydroxymethyl)phenylglyoxylic Acid (14). **35** (4.5 g, 0.018 mol) was added to a solution of NaOH (3.0 g, 0.075 mol) in water (30 mL), and the solution was warmed on a steam bath for 1 h, cooled, and then washed with Et_2O (50 mL); the aqueous layer was then acidified with concentrated HCl and extracted with Et_2O (2 × 75 mL), and the combined organic extracts were washed, dried, and evaporated to give **14** as a cream solid: yield 1.6 g (49%); mp 119–120 °C from EtOAc/hexane. Anal. ($C_9H_8O_4$) C, H.

4-Hydroxyphenylglyoxylic Acid (11). DL-2-(4-Hydroxyphenyl)glycine (75.15 g, 0.45 mol) was added portionwise with stirring to trifluoroacetic anhydride (210 mL, 1.5 mol), and the mixture was heated at reflux for 2 h. The solvent was then removed by distillation under reduced pressure, and the residual oil was allowed to cool and solidify to give the oxazolone (**6**), mp 46–49 °C. The oxazolone was then added to a stirred solution of NaOH (60 g) in H_2O (250 mL), and the resulting solution was stirred at room temperature for 4 h. After filtration to remove unwanted solids, the filtrate was acidified to pH 2 with concentrated HCl, whereupon the product (**11**) precipitated as the sodium salt and was filtered off and carefully washed with H_2O to remove NaCl contaminant. The sodium salt was suspended in water (200 mL) and acidified with concentrated HCl to pH 1. The resultant thick paste was digested with Et_2O, and the ethereal solution was washed, dried, and evaporated to give a semicrystalline solid. Trituration afforded a solid (**11**), which recrystallized from Et_2O/40–60 °C petroleum ether: yield 52 g (70%); mp 174–176 °C (lit.[10] mp 177.5–178 °C).

Compounds **21** and **22** were also prepared by this method starting from the appropriate amino acids.

4-Hydroxy-3,5-diiodophenylglyoxylic Acid (23). Iodine monochloride (3.4 mL ≡ 10.03 g) was added dropwise over a period of 0.5 h to 4-hydroxyphenylglyoxylic acid (**11**; 5.00 g, 0.03 mol) in AcOH (10 mL). The mixture was heated at 60 °C for 1 h, cooled, and evaporated to dryness, and toluene was added to the resultant solid. The toluene solvent was evaporated off, the residual pink solid was treated with hot H_2O, and the resulting mixture was filtered. A few drops of concentrated HCl were added to the filtrate and a white precipitate of the product appeared over a period of 48 h. This was recrystallized from water to give the 3,5-diiodo product **23**: yield 0.55 g (9%); mp 162–163 °C (lit.[37] mp 164–166 °C).

DL-2-[4-(Methylthio)phenyl]glycine (29). α-Hydroxyhippuric acid (9 g, 0.046 mol) was added to a solution of concentrated H_2SO_4 (4 mL) in AcOH (36 mL) according to the method of Ben-Ishai, Satati, and Berler.[15] Thioanisole (6.3 g, 0.05 mol) was added, and the mixture was stirred overnight at room temperature and then added to ice–water. The resulting mixture was extracted with EtOAc (2 × 150 mL), and the organic extracts were dried and concentrated to half volume. The EtOAc solution was then extracted with aqueous Na_2CO_3 solution, the aqueous layer was separated and acidified, and the precipitate was filtered off to give DL-*N*-benzoyl-2-[4-(methylthio)phenyl]glycine: yield 10.5 g (70%); mp 173–174 °C. The *N*-benzoyl compound (6.0 g) was treated with concentrated HCl (15 mL) in H_2O (10 mL), and the mixture was vigorously stirred and heated at reflux for 4 h and then allowed to cool. The small amount of starting material was filtered off, and the filtrate was basified with NaOH and then carefully reacidified to pH 4. The product was filtered off, washed with H_2O, and crystallized from H_2O/EtOH: yield 1.6 g (41%); mp 242–244 °C (lit.[41] mp 230–231 °C).

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The phenylglycines in Table I were prepared by this general method prior to resolution into L-(+) and D-(-) isomers. Resolution of the isomers was achieved by selective enzymatic degradation using hog kidney acylase.

L-(+)-2-(4-Hydroxyphenyl)glycine (25). DL-2-(4-Methoxyphenyl)glycine (93 g, 0.514 mol) was suspended in H_2O (1.3 L) and to the stirred suspension was added NaOH (21.3 g, 0.514 mol). Chloroacetic anhydride (177 g, 1.027 mol) was then slowly added over a period of 0.5 h, with cooling, followed by further addition of NaOH (42.6 g) in H_2O (200 mL). The pH was maintained at 9 by addition, if necessary, of more NaOH solution, and stirring at room temperature was continued for a 1.5 h period. The solution was then acidified to pH 2 by addition of concentrated HCl, and the resultant pale yellow precipitate of DL-*N*-(chloroacetyl)-2-(4-methoxyphenyl)glycine was filtered off, washed, and dried: yield 51 g; mp 174-178 °C (lit.[40] mp 182-183 °C).

The DL-*N*-(chloroacetyl)-2-(4-methoxyphenyl)glycine (51 g) was suspended in distilled H_2O (770 mL) and to this was added sufficient NH_4OH to maintain the pH at 7.8 and effect solution. Hog kidney acylase enzyme (2.8 g) (acylase-1, Sigma Chemical Co.) was added, and the solution was stirred at 37 °C for 22 h. A light brown precipitate of **27** was obtained and filtered off [the filtrate contains D-(-)-*N*-(chloroacetyl)-2-(4-methoxyphenyl)-glycine]. The crude **27** was added to hot 3 N HCl (100 mL) containing charcoal, and the mixture was warmed gently and filtered. The cooled filtrate was treated with 0.880 ammonia until pH 5-6 was obtained. A white crystalline solid of L-(+)-2-(4-methoxyphenyl)glycine (**27**) precipitated and was filtered off and recrystallized from water: yield 7.7 g (43%); mp 218 °C dec; $[\alpha]^{25}_D$ +137° (lit.[40] $[\alpha]^{25}_D$ +150.4°).

Compound **27** (10 g) was added to 48% HBr (100 mL), and the mixture was heated under reflux with stirring for 5 h. The resultant red solution was evaporated to dryness; the residue was then treated with H_2O (50 mL) and the mixture filtered. The filtrate was brought to pH 5 by addition of 0.880 ammonia, whereupon a solid precipitated after cooling. The solid was filtered off, washed, and recrystallized from H_2O to give **25**: yield 5.5 g (60%); mp 230 °C dec; $[\alpha]^{25}_D$ +124.5° (lit.[39] mp 225 °C dec).

D-(-)-2-(4-Methoxyphenyl)glycine (28). D-(-)-*N*-(chloroacetyl)-2-(4-methoxyphenyl)glycine (64 g) obtained as described above was added to 2 N HCl (680 mL), and the mixture was stirred and heated under reflux for 1.5 h. The solution was filtered while still hot and cooled, and the pH was adjusted to 5.5 using 0.880 ammonia. The resulting solution was cooled for a further 2 h, and then the precipitate was collected, washed, and recrystallized from H_2O to give **28**: yield 30 g (67%); mp 219-220 °C; $[\alpha]^{25}_D$ -140° (lit.[40] $[\alpha]^{25}_D$ -149.9°).

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4-Amino-4-arylcyclohexanones and Their Derivatives: A Novel Class of Analgesics. 2. Modification of the Carbonyl Function

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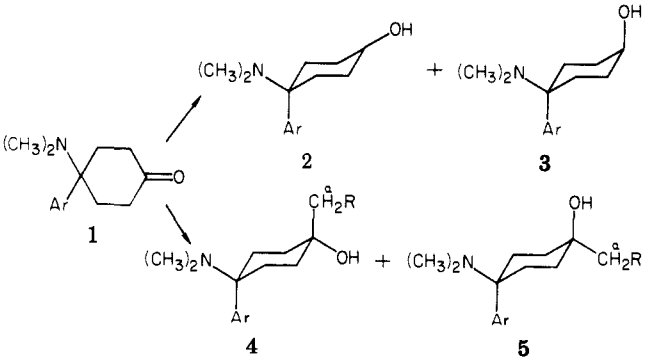
The effect on potency of modification of the carbonyl function of analgesics derived from 4-(dimethylamino)-4-arylcyclohexan-1-one was studied by reduction and by addition of nucleophiles. The resulting amino alcohols were separated and assigned structures on the basis of X-ray crystallography, NMR, and TLC mobility. The trans (OH and N) isomers were invariably more potent than the cis. Inclusion of flat lipophilic moieties (phenyl, cyclohexenyl) at a distance of at least two carbon atoms from the carbon bearing hydroxyl led to increases in potency by orders of magnitude. The possible significance of this on receptor interaction is discussed.

In the first report[2] in this series, we described the effect of substitution on the aromatic ring on the analgesic potency in a series of 4-(dimethylamino)-4-arylcyclohexanones and their ketals. The observation of marked differences in potency between these compounds and those lacking the oxygen indicated that the oxygen function at the 1 position has an important role in the activity of this series. In the present work, we describe the SAR of a series of derivatives in which nucleophiles have been added to the carbonyl function.

Chemistry. Reduction of ketones 1 (Ar = C_6H_5) by means of $NaBH_4$ afforded a pair of isomeric alcohols in the ratio of 4:1. The finding that these showed enhanced analgesic potency over the corresponding ketones and ketals[2] led us to extend this series to organometallic adducts of the carbonyl group. Condensations were all carried out with a large excess of reagents (RLi or RMgBr); though reactions were allowed to proceed as long as 3 days, considerable amounts of starting ketones were invariably recovered. In contrast to the stereoselectivity observed in the reduction, condensations afforded roughly equal amounts of isomeric amino alcohols. These, however, exhibited sufficiently different polarities on silica gel to make them easily separable.



Assignment of configuration of the reduction products (**2** and **3**) by NMR was relatively straightforward. Both chemical shift and multiplicity of the carbon-bearing oxygen confirmed our expectation that the predominant isomer bore an equatorial hydroxyl group. Some of our

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