33. Stereoselective *in-vitro* Aromatic-Ring Oxygenations of chiral 1,4-Benzodiazepin-2-ones

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Dedicated to Professor Dr. *Vladimir Prelog* on occasion of his 70th birthday

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Summary

Biological N(1)-demethylation and C(3)-hydroxylation of two enantiomeric 1,4-benzodiazepin-2-ones **1** and **2** (cf. *scheme 2*) were found to be nonstereoselective. Aromatic-ring hydroxylation, however, took place in the (*S*)-series only, leading to 3′- and 4′-hydroxylated, N(1)-demethylated, metabolites (**54** and **56**, cf. *scheme 5*: these structures were unambiguously confirmed by comparing their UV., CD., and mass spectra with those of authentic specimens). Several compounds, theoretically conceivable as products of hydroxylation in the aromatic A-ring of **1** and **2** by mechanisms including the NIH-shift (cf. *scheme 3*), were synthesized but none of these compounds was yet isolated from *in vitro* incubation mixtures.

Introduction. – Compounds in the 1,4-benzodiazepin-2-one series are known as potent tranquilizing and anticonvulsant agents [1] [2]. Their pharmacology and biotransformations were, therefore, extensively studied [3–5], and still these fields are of major interest. Another actively investigated field regarding these compounds is that of structure-activity relationships, especially the connection of their biological effects and stereochemistry. Because of the complex architecture of these molecules – a factor particularly affecting the conformation of the non-aromatic seven-membered ring, which may set a limit to the free rotation of the C(5)-phenyl group – however, no final conclusions can be reached as to the relationship of dynamic stereochemical properties and biological activity of this type of structure.

Some authors have intimated that stereoselective biological action may be considered as a likely cause of the phenomena observed after administration of racemic

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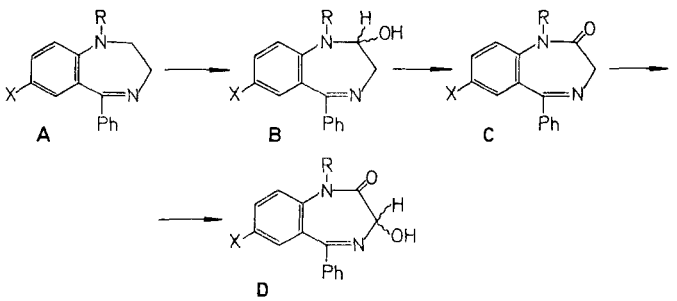
mixtures of chiral 3-monosubstituted 1,4-benzodiazepines [6] [7]. Stereoselectivity in biotransformation of these compounds, again, was considered as especially likely by other authors [8–11]. A few general remarks on stereoselective processes in biotransformation of 1,4-benzodiazepines, achiral and chiral, seem, therefore, to be well justified as an introduction.

All achiral 1,4-benzodiazepines contain some prochiral group in the non-aromatic ring. The methylene group in 3-position is such a group, in the first place. Other prochiral groups are: the planar azomethine double bond, and the carbonyl group in 2-oxo derivatives. The methylene group in 3-position carries a pair of enantiotopic ligands, whereas the other two prochiral groups possess enantiotopic faces [12] [13]. Stereospecific enzymes able to convert prochiral precursors into one of the two possible chiral products may discriminate between such faces.

Some of the known steps in biotransformation of 1,4-benzodiazepines are, in fact, distinguishable stages of a multistep process, like that shown below for oxygenations and oxidations at positions C(2) and C(3):

Scheme 1



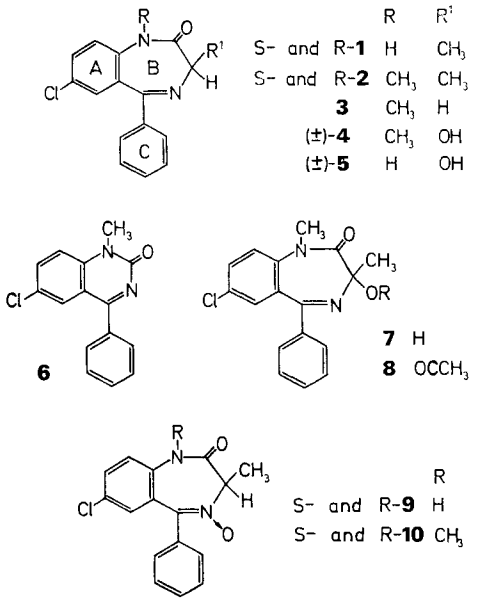
Whenever a formation or conversion of only one of the enantiomers can be demonstrated, the above steps may be termed as product- or substrate-stereoselective, respectively, according to *Prelog*'s nomenclature of enzymic reactions [14]. In particular, steps A → B and C →D are product-stereoselective, step B → C, in contrast, is substrate-stereoselective.

In oxygenation of either aromatic ring of 1,4-benzodiazepines, which is a frequent biotransformation pathway with many similar substrates [15–17], only a substrate-stereoselective process should be expected to occur with chiral substrates.

The most often encountered *in-vivo* biotransformations of 1,4-benzodiazepines include N(1)-dealkylation, hydroxylation of the hetero- and homocyclic rings, hydrolysis of lactam and azomethine bonds, N(4)-oxidation, and formation of C(3)-O-glucuronides [5] [18]. The actual pathway taken by a particular derivative depends on the substrates bound in various positions of its basic frame.

Results and Discussion. – To prepare ourselves for a continuation of investigations started earlier [19] [20], we have systematically studied stereoselectivity in biotransformations of the enantiomeric pairs of **1** and **2**, by liver enzymes *in vitro*. Our first observations giving evidence as to stereoselectivity were presented in a preliminary report [8]. At that time we had shown dependence of aromatic-ring hydroxylation

Scheme 2



on configuration, but we found at the same time that hydroxylation in 3-position, as well as N(1)-demethylation, were non-stereoselective with either configuration *S*- or *R* of **2**. Typical tin layer chromatograms (TLC.) of extracts obtained from incubation mixtures containing the hepatic preparation and (*S*)- and (*R*)-**1** and (*S*)- and (*R*)-**2** are reproduced in Fig. 1. Compound (*S*)-**1** gave rise to two metabolites separable by

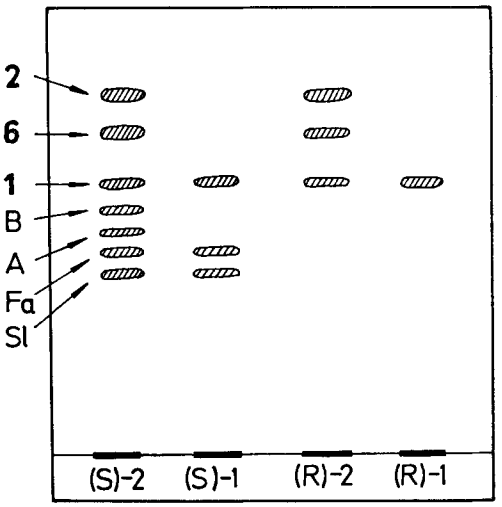


Fig. 1. *Typical TLC. of metabolites resulting from conversion of* (S)- *and* (R)-*configurations of compounds* **1** *and* **2** *by rat-liver enzymes* (Fa = faster metabolite, Sl = slower metabolite, A and B = their N(1)-methyl-derivatives)

preparative TLC., whereas (*S*)-**2** gave five separable metabolites. The Rf-value and the UV. spectrum of **1** [a descendant of (*S*)-**2**] indicated that this biotransformation product was identical with authentic (*S*)-**1**, which was confirmed by agreement of CD. and mass spectra. The main metabolite of (*R*)-**2** was identified as the N(1)-demethyl derivative (*R*)-**1**, because both gave superimposable UV., CD., and mass spectra. These results mean that N(1)-demethylation of the chiral, (*R*)- and (*S*)-**2** was non-stereoselective. In similar experiments with (*R*)-**1** no biotransformation whatsoever could be observed.

Both configurations of **2** (*S*- and *R*-), as well as the prochiral compound **3** (7-chloro-1-methyl-5-phenyl-2*H*-1,3-dihydro-1,4-benzodiazepin-2-one, generic name: *diazepam*), gave a common metabolite characterized by a molecular ion, $m/e = 270$, on mass spectra. All spectral properties of this metabolite pointed towards a structure such as given by formula **6** [21]. To clarify whether **6** is formed by enzyme action or otherwise, compounds (*S*)-**2**, (*R*)-**2**, **3**, **4** (7-chloro-3-hydroxy-1-methyl-5-phenyl-2*H*-1,3-dihydro-1,4-benzodiazepin-2-one, generic name: *temazepam*), and **7** were incubated separately, without addition of the hepatic enzyme preparation. In these experiments we could isolate compound **6** from the incubation mixtures with either of the two 3-hydroxy derivatives **4** and **7**, but we failed to isolate **6** from mixtures with the 3-dehydroxy compounds (*S*)-**2**, (*R*)-**2**, and **3**. Thus, **6** is formed by non-enzymatic rearrangement of 3-hydroxy intermediates which, in turn, arise from enzymatic hydroxylations of (*S*)-**2**, (*R*)-**2**, and **3**. Compound **7**, in contrast, was obviously formed by *enzymatic* hydroxylation of (*S*)-**2** and (*R*)-**2**, which reveals that C(3)-hydroxylation of *chiral* 3-monosubstituted 1,4-benzodiazepines is not stereoselective[2]).

Aromatic-ring hydroxylation resulted in two isomeric products, as was concluded from their mass, UV., and CD. spectra, but thorough mass-spectrometric investigation [23] failed to give a definitive answer to the question about the site of hydroxylation. This failure is particularly due to the fact that the 7-chlorine atom splits off early in the fragmentation pathway, which gives a number of fragments that do not allow identification of the original rings A and C. Interestingly enough, earlier *in-vitro* studies with 1,4-benzodiazepines by other authors gave merely indication of N(1)-demethylation and C(3)-hydroxylation: as no other aromatic-ring hydroxylations were observed, the metabolites hydroxylated in their aromatic rings were assumed to originate from extrahepatic conversion [24].

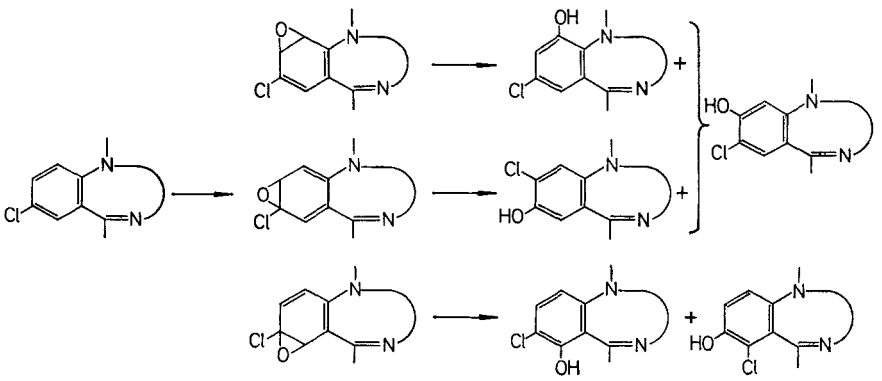
Under different circumstances hydroxylations of aromatic and aliphatic compounds are known to be catalysed by hepatic mixed-function oxygenases [25–27]. Hepatic monooxygenases convert aromatic compounds into arene oxides, and these undergo spontaneous isomerization by the so-called NIH-shift [28–31]. The isomerization is often accompanied by the loss or migration of a proton, a deuteron, a halogen atom, or an alkyl substituent [30]. Formation of arene oxides is followed by hydro-

[2]) This is the opposite to what is found by isotopic labeling, when *prochiral* $H_2C(3)$ is hydroxylated enzymatically (see [11]). Stereoselectivity of C(3)-hydroxylation of **3** labeled in 3-position was thus unambiguously established. Yet, when we repeated this experiment with unlabeled **3**, the 3-hydroxy derivatives isolated, namely **4** and **5**, exhibited no circular dichroism, that is, they were racemic. We found later [22] that the optically inactive compounds **4** and **5** arise from *in-vitro* racemization, without C(3)–H/D exchange, presumably by ring-chain tautomerism.

xylation in *o*- and *p*-positions, whereas *m*-hydroxylated isomers usually arise by direct oxidation, presumably by oxygen insertion [25] [31].

In agreement with the mechanism of NIH-shift, formation of the intermediate arene oxides, and of products hydroxylated in the A-ring of 1,4-benzodiazepines might take the pathway outlined in *Scheme 3*:

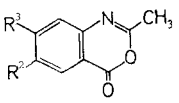
Scheme 3



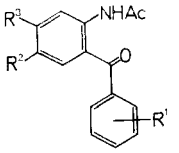
Two additional isomers might arise by *o*- and *p*-hydroxylation, respectively, of ring C, whereas oxygen insertion into its *m*-position [25] might produce one more hydroxylated isomer. To determine the position of hydroxyl groups in isolated hydroxylated metabolites of (*S*)-**1** unequivocally, an extensive synthetic program was elaborated, providing for preparation of likely hydroxylated products and making them available for comparison to the metabolites actually isolated. This way was chosen particularly in view of repeatedly expressed criticism of previous work on metabolic products of benzodiazepines (*cf.* [32]). Results reported in the past were regarded as possibly erroneous because GLC./MS. determination used in such studies causes thermal modifications, which may produce artifacts on the mass spectra, and therefore it is possible that results obtained by mass spectroscopy were misinterpreted in the earlier literature.

The last two steps in the synthesis of possible metabolites, that is, preparation of the 2-[(*N*-carbobenzoxy-(*S*)-alanyl)-amino]-chloro-hydroxy-benzophenones **43–50** (cf. *scheme 4*) followed by removal of the protecting group and cyclization into the corresponding compounds **53–61** (cf. *scheme 5*) are equivalent to the procedure described in one of our previous publications [19]. Preparation of some of the poly-substituted benzophenones, however, required multi-step synthetic procedures starting from commercially available materials. Thus, A-ring trisubstituted benzophenones **40** and **41** were prepared according to *Schemes 6* and *7*, respectively. The four differently substituted benzophenones, **37–39** and **42**, were prepared by published procedures, but in some instances the yields were markedly improved, and all products were identified unequivocally, which had not been done by authors of the original publications (see experimental part for details).

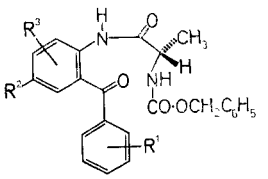
Scheme 4



	R^2	R^3
24	Cl	OCH_3
25	OCH_3	Cl
26	Cl	H

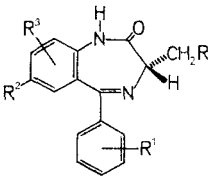


	R^1	R^2	R^3
27	2′-OCH_3	Cl	H
28	3′-OCH_3	Cl	H
29	4′-OCH_3	Cl	H
30	H	OCH_3	Cl
31	H	Cl	OCH_3



	R^1	R^2	R^3
43	2′-OH	Cl	H
44	3′-OH	Cl	H
45	4′-OH	Cl	H
46	4′-OCH_3	Cl	H
47	H	OH	4-Cl
48	H	OH	4-OH
49	H	Cl	3-OH
50	H	Cl	H

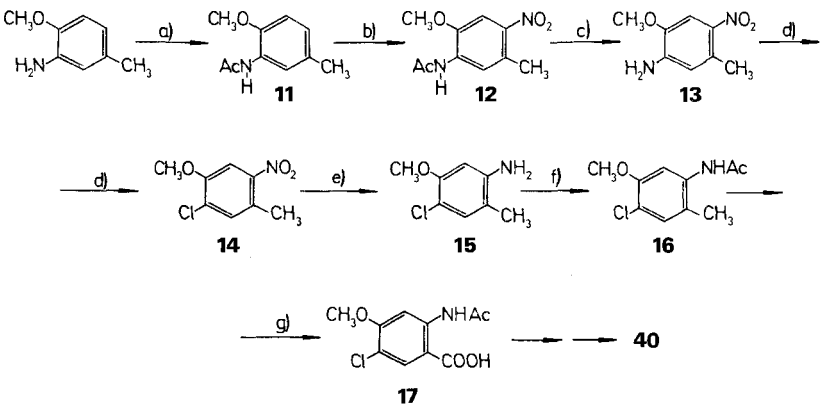
Scheme 5



	R	R^1	R^2	R^3
51	OH	H	Cl	H
52	OCa*	H	Cl	H
53	H	2′-OH	Cl	H
54	H	3′-OH	Cl	H
55	H	3′-$OCOCH_3$	Cl	H
56	H	4′-OH	Cl	H
57	H	4′-OCH_3	Cl	H
58	H	H	OH	8-Cl
59	H	H	Cl	8-OH
60	H	H	Cl	9-OH
61	H	H	H	H

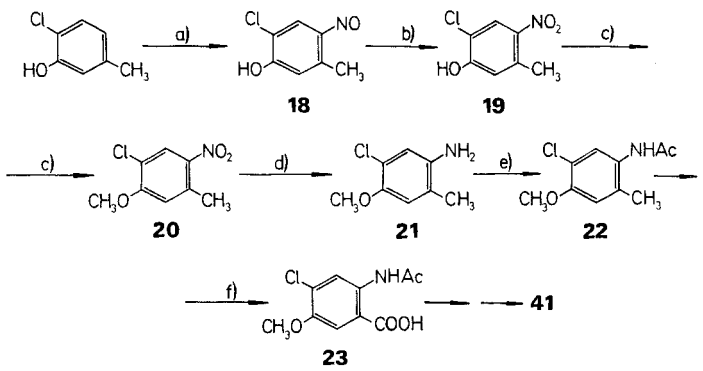
* OCa = O-camphoyl

Scheme 6



a) $Ac_2O/60°$; b) HNO_3 in $AcOH/H_2SO_4$; c) 40% NaOH/80°; d) $NaNO_2$ in $AcOH/H_2SO_4$ + Cu_2Cl_2 in HCl aq. (1:1); e) Sn/HCl conc.; f) $Ac_2O/60°$; g) $KMnO_4$ in aq. $MgSO_4$.

Scheme 7



a) $NaNO_2$/dil. NaOH; b) 23% HNO_3/40°; c) $(CH_3)_2SO_4$/10% KOH-solution; d) RaNi/H_2; e) Ac_2O/60°; f) $KMnO_4$ in aq. $MgSO_4$.

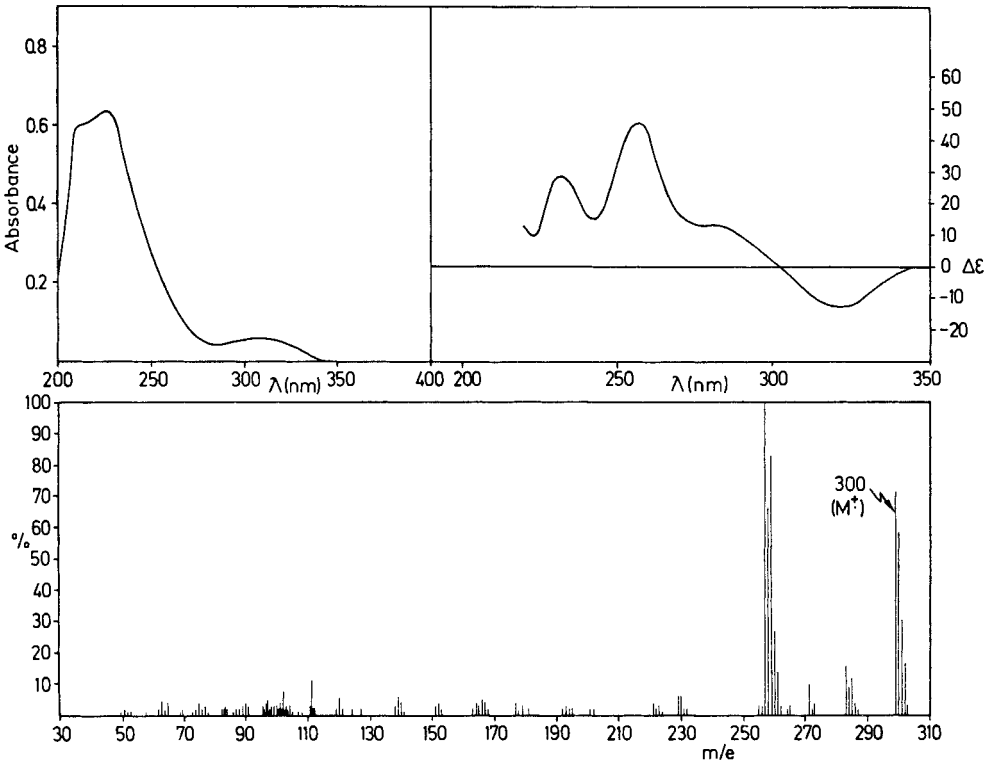


Fig. 2. *UV., CD., and mass spectra of compound* **54**: these spectra are superimposable on corresponding spectra of slower-moving metabolite (*cf.* Fig. 1).

Racemic **51**, prepared in the course of this program, was tentatively subjected to separation of its enantiomers *via* their camphanic esters. All attempts to separate the diastereomeric esters by crystallization or by column chromatography on silica failed, however, despite of successful application of these procedures in separation of diastereomeric 3-*O*-camphoyl-1,4-benzodiazepines in our earlier work [7].

Hydroxylated 1,4-benzodiazepines **4**, **7**, **56** and **60** and the *O*-methylated derivative **57** were the first to be prepared because *in-vivo* studies by some authors [5] [33] [34] had shown that certain prochiral 3-monosubstituted 1,4-benzodiazepines are oxygenated in positions 9 and 4′ (in one instance a 4′-methoxy derivative was identified [21]). Comparison of UV. CD. and mass spectra of **56**, **57** and **60** to those of the two unknown biotransformation products established the identity of one of them with the 4′-hydroxy derivative **56**. Further comparison of spectra indicated that the other biotransformation product was not identical with either of compounds **51**, **53**, **58**, or **59**; however, the spectra of this unknown metabolite were superimposable on those of the 3′-hydroxy derivative **54**, which settles the question of identity of the second biotransformation product.

According to the mechanism now accepted for aryl oxygenations [25] [31], the 3′-hydroxylated metabolite may be formed *via* one of two possible arene oxides, that

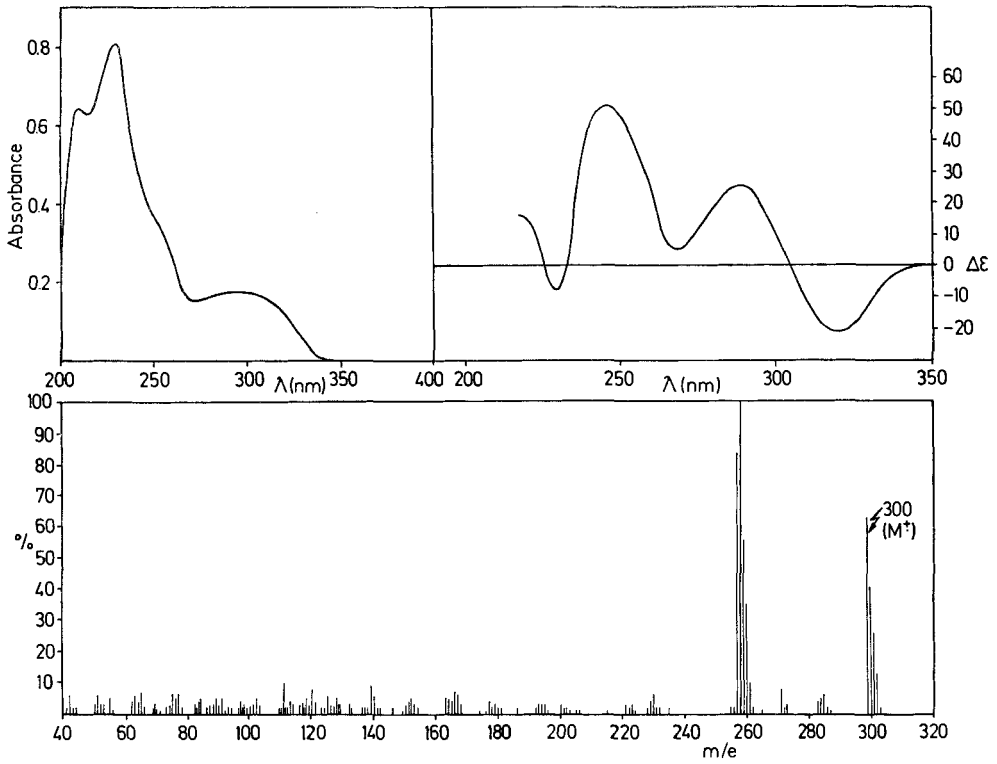


Fig. 3. *UV., CD., and mass spectra of compound* **56**: these spectra are superimposable on corresponding spectra of faster-moving metabolite (*cf.* Fig. 1).

may rearrange because of the slight electron-withdrawing effect due to the azomethine group. Such rearrangements leave the oxygen atom in 3'-position. Alternatively, 3'-hydroxylation may occur by an insertion of oxygen atom. We cannot, as yet, decide between these alternatives, but hope being able to do so after further studies intended, when we shall use C(5)-phenyl-tritium labeled compounds[3]). Thus, the results presented in this paper suggest that *in-vitro* action of either of the two oxygenases, catalysing mechanistically different oxidation processes, might be stereoselective with the (*S*)-configurations of both **1** and **2**. To the best of our knowledge, moreover, our experiments have provided the first instance where a liver-enzyme catalysed *m*-oxygenation was observed *in vitro*. Observation of *m*-oxygenation *in vivo* was, of course, previously reported in the literature [35].

Detailed investigations of stereoselective *in-vivo* biotransformations of various chiral 3-monosubstituted 1,4-benzodiazepines are now in progress in our laboratories.

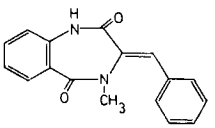
Experimental Part

Enzyme preparations. – Rat liver tissue was obtained from adult male animals kept fasting for 24 h after a pretreatment with phenobarbital [three doses (40 mg/kg intraperitoneally, or 80 mg/kg orally) given once daily for three consecutive days], as used by others [31] to induce aromatic-ring-hydroxylation enzymes. Animals were killed by decapitation, the organs were rapidly excised, rinsed with iced water, weighed, and homogenized for 60 s in two volumes of ice-cold 0.2M *tris*(hydroxymethyl)methylamine-HCl buffer pH 7.4 (tris buffer) using an *Edmund Bühler* homogenizer at $2 \cdot 10^4$ rev./min. The resulting homogenate was centrifuged at 10^5 *g* for 0.5 h to obtain a postmitochondrial supernatant containing microsomes. Further centrifugation at 10^5 *g* for one h yielded a microsomal pellet. Postmitochondrial supernatants and microsomal pellets were either used immediately for incubation, or they were stored at $-20°$.

Incubation in vitro. – Hepatic protein preparations (postmitochondrial supernatants, microsomes) were supplemented with pyridine nucleotides, energy supplier, salts, and an NDPH-generating system[4]), and were incubated with substrate (chiral 3-monosubstituted 1,4-benzodiazepines) either as 12-ml samples in 25-ml flasks – for monitoring purposes – or as 300-ml samples in one-liter flasks for isolation and structure determination of metabolites.

The 12-ml samples consisted of 1.2 ml of hepatic protein preparation (corresponding to 0.4 g of fresh tissue), 0.8 ml of 0.01M ATP, 0.8 ml of 0.03M glucose-6-phosphate, 0.4 ml of 0.03M NAD, 0.4 ml of 0.03M NADP, 0.4 ml of 2M KCl, 0.4 ml of 0.1M $MgCl_2$, 2.8 i.u. of glucose-6-phosphate-

[3]) Recently *Luckner et al.* (Eur. J. Biochem. *37*, 78 (1973)) reported *m*-oxygenation of 3-benzylidene-1,4-benzodiazepin-2,4-dione (dehydrocyclopenin, **I**), proceeding presumably *via* an epoxide (cyclopenin) to give a *m*-hydroxylated product (cyclopenol, **II**), but the actual sequence of steps was not unequivocally ascertained. Nevertheless, if an NIH-shift were the mechanism leading to aryl hydroxylation, one should assume that the epoxide ring must exert a *m*-directing influence.



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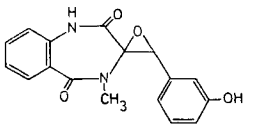


II

We are indebted to Professor *G. W. Kirby*, Department of Chemistry, University of Glasgow, for having brought *Luckner*'s papers to our attention.

[4]) All coenzymes and the NDPH-generating enzyme (G-6-P dehydrogenase) were purchased from *Boehringer Sons*, Mannheim.

dehydrogenase, and 0.8 ml of a solution containing 2.8 μmol of substrate. This incubation mixture was made up to a final volume of 12 ml with 0.2M *tris* buffer pH 7.4. Blanks contained all ingredients except substrate. Additional blanks were run omitting the hepatic protein preparation to correct for possible nonenzymatic conversion of substrate. All samples were incubated in a '*New Brunswick*' shaker-incubator Mod. G 76 at 37° for a 4-h period.

Isolation and analysis of metabolites. – 300 ml samples of incubation mixture having the same composition as the 12-ml samples described in the preceeding paragraph were agitated 4 h at 37°, then extracted three times with 600-ml portions of ethyl acetate. The combined extracts from one batch were evaporated to dryness, and the residue was dissolved in a small volume of ethanol. A little ethanolic solution was spotted on TLC. plates to try out various solvent systems for separation of components. Detection and location of the latter was made by UV. (254 nm) illumination. The bulk of ethanolic solution was applied to 20 × 20-cm prep. TLC. plates, and treated with the proper solvent systems found by previous TLC.-examination. Areas containing the metabolites were scraped off the plate and eluted three times with ethanol. Each metabolite was purified by repeated prep. TLC. Part of the purified metabolites was used to record UV., CD., and mass spectra. Of the remaining part, 0.5 to 1.0-mg samples were taken to hydrolyse conjugates by 4 h's heating at 100° with 6M HCl. After neutralization with sodium hydroxide, the resulting products were extracted with ether, separated by TLC., and purified by several runs of prep. TLC. UV., CD., and mass spectra were compared to those of synthetically prepared compounds representing theoretically possible metabolites.

Chromatography. – Plates precoated with *Merck*'s silica gel 60F254 were used in all runs. Layer thicknesses were: 0.25 mm for TLC., and 2 mm for preparative TLC.

Spectra[5]**).** – UV. absoption spectra were recorded with a *Varian* Techtron M 635 spectrophotometer using samples dissoved in either 96% ethanol, or in 0.1M HCl. Spectra recorded with isolated metabolites were considered to merely represent qualitative absorbance *vs.* wavelength profiles, but to be entirely satisfactory for comparison with spectra of the synthetic products.

IR. spectra (KBr pellets) were obtained with a *Perkin Elmer* M 257 spectrophotometer (absorptions in cm^{-1}).

CD. spectra of samples dissolved in 96% ethanol were traced with a *Japan Spectroscopic Company* Model J-20 spectropolarimeter using 10-mm cells. Instrument responses were recalculated to express molar ellipticities (deg cm^2 $dmol^{-1}$), but again, CD. spectra of metabolites have only qualitative significance and serve only for comparison with those of authentic samples.

Mass spectra were recorded with a *CEC* 21-110 C spectrometer at 70 eV. The direct insertion technique was used for sample application (see *Rendić et al.* [23] for details).

NMR. spectra were run on a *Varian* T-60 instrument with TMS serving as an internal standard. Signal positions are given in ppm on the δ-scale (abbreviations used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br. = broad). Coupling constants are given in Hz.

Synthetic Part. – *General.* Melting points (m.p.) were determined on a *Kofler* microheating stage (*Boëtius*) and are given as uncorrected values for all compounds. Optical rotations were measured either on a *Perkin Elmer* M 141 spectropolarimeter (at $\lambda = 578$ nm), or on a *Zeiss* polarimeter at λ_D. Samples intended for rotation measurements, as well as for microanalyses, were dried at 0.01 Torr (ambient temperature). Column chromatographic purification procedures were carried out with silica gel (grain sizes 0.05–0.2 mm) from *Merck*. Effluent fractions were collected with an *LKB* 7000 automatic fraction collector, and the composition of particular fractions was monitored by TLC. Solvent evaporations were always made under reduced pressure in a Rotavapor (RV.) (*Büchi*) apparatus. (RT. = roomtemperature).

Synthetic procedures used to prepare the two enantiomers of chiral compounds from corresponding precursors were the same for a particular pair. All compounds described in subsequent paragraphs are labeled with the numbers they carry in schemes (the sequence of numbers appearing in the text being, therefore, necessarily out of natural order in several places).

[5]) Permissions to use facilities for recording CD. spectra and mass spectra at the Laboratory of Macromolecular Biophysics, Institute of Biology, University of Zagreb, and the Laboratory of Mass Spectroscopy, Institute 'Jožef Štefan', Ljubljana, respectively, are gratefully acknowledged.

(*3S*)-*7-Chloro-3-methyl-5-phenyl-2*H-*1,3-dihydro-1,4-benzodiazepin-2-one-4-oxide* ((*S*)-**9**). (3*S*)-7-Chloro-3-methyl-5-phenyl-2*H*-1,3-dihydro-1,4-benzodiazepin-2-one ((*S*)-**1**, 1.45 g, 5.1 mmol) was gently heated in 17.5 ml of glacial acetic acid until all dissolved. 10 ml of 30% H_2O_2 was then added – at ambient temperature – over a 12-h period. The reaction mixture was poured into 100 ml of ice/water, and the products were extracted with ether (3 × 70 ml). The ether extracts were combined, washed with 2% NaOH-solution (2 × 70 ml), then with water (2 × 50 ml), dried, evaporated, and the residual oil was applied to a chromatographic column (40 g of silica gel). The desired product was eluted with ether, collecting 10-ml portions of the eluate. Fractions 15–27 contained 980 mg of crude **9**. This product was recrystallized from chloroform/ether: m.p. 284–286°, $[\alpha]_{578} = +1.35°$ ($c = 1.52$, DMSO). – NMR. (DMSO-d_6): 1.48 (*d*, $J = 6.4$, 3H); 4.56 (*q*, $J = 6.4$, 1H); 6.9–7.7 (*m*, 8H); 11.14 (br. *s*, 1H).

$C_{16}H_{13}ClN_2O_2$ (300.74) Calc. C 63.91 H 4.35 N 9.32% Found C 64.20 H 4.65 N 9.42%

(*3R*)-*7-Chloro-3-methyl-5-phenyl-2*H-*1,3-dihydro-1,4-benzodiazepin-2-one-4-oxide* (*R*)-**9**) M.p. 283–285°, $[\alpha]_{578} = -1.29°$ ($c = 1.50$, DMSO).

(*3S*)-*7-Chloro-1,3-dimethyl-5-phenyl-2*H-*1,3-dihydro-1,4-benzodiazepin-2-one-4-oxide* [(*S*)-**10**]. To a solution of (*S*)-**2** (2.05 g, 6.85 mmol) in methylene chloride (40 ml), 3.0 g (17.5 mmol) of *m*-chloroperbenzoic acid (wet-stabilized, *Aldrich* pract.) dissolved in methylene chloride (20 ml) was added dropwise with stirring. After additional stirring for 3 h at RT. the reaction mixture was dried, evaporated, the residue applied to a column (60 g of silica gel), and the desired product was eluted with ether collecting 10-ml portions of eluate. Fractions 22–40 contained 1.76 g of an oily product which crystallized from cyclohexane: m.p. 83–85°, $[\alpha]_{578} = +30.3°$. – NMR. ($CDCl_3$): 1.67 (*d*, $J = 7$, 3H); 3.52 (*s*, 3H); 4.46 (*q*, $J = 7$, 1H); 7.1–7.9 (*m*, 8H).

$C_{17}H_{15}ClN_2O_2$ (314.77) Calc. C 64.88 H 4.80 N 8.89% Found C 65.05 H 5.02 N 8.90%

(*3R*)-*7-Chloro-1,3-dimethyl-5-phenyl-2*H-*1,3-dihydro-1,4-benzodiazepin-2-one-4-oxide* [(*R*)-**10**]. M.p. 82–85°, $[\alpha]_{578} = -30.7°$ ($c = 1.02$, $CHCl_3$).

rac.-*7-Chloro-1,3-dimethyl-3-acetoxy-5-phenyl-2*H-*1,3-dihydro-1,4-benzodiazepin-2-one* (**7**). Compound (*S*)-**10** (1.57 g, 5.0 mmol) was heated in 20 ml of acetic anhydride, and worked up as described by other authors [36]. Isolated crude **7** (1.1 g, 61.5%) had $[\alpha]_{578} = 0 \pm 0.1°$, and, after crystallization from chloroform/light petroleum, m.p. 154–155° ([36]: 150–152°). – NMR. ($CDCl_3$): 1.36 (*s*, 3H); 2.16 (*s*, 3H); 3.48 (*s*, 3H); 7.1–7.8 (*m*, 8H).

rac.-*7-Chloro-1,3-dimethyl-3-hydroxy-5-phenyl-2*H-*1,3-dihydro-1,4-benzodiazepin-2-one* (**8**). Hydrolysis of **7** (0.75 g, 2.1 mmol) in conc. sulfuric acid (10 ml) gave crude **8** (0.56 g, 84.7%), m.p. 154–157° which, on crystallization from chloroform/light petroleum, rose to 158–159° ([36]: 156–158°).

3-Acetylamino-4-methoxytoluene (**11**). 3-Amino-4-methoxytoluene 10.0 g, 73 mmol (*Fluka*, purum) was acylated with acetic anhydride (15 ml) at 60° for 1 h under stirring. Dilution with water (150 ml) caused a formation of crystals, which were collected and dried: 12.7 g (98%) of pure **11** was obtained, m.p. 108–110° ([37]: 110°).

2-Nitro-4-methoxy-5-acetylaminotoluene (**12**). Compound **11** (11.0 g, 61.5 mmol) was dissolved in a mixture of glac. acetic acid (15 ml) and sulfuric acid (20 ml), and nitrated at 15–20° by dropwise addition of nitric acid (3.0 ml, $d = 1.48$) with stirring. After additional stirring for 0.5 h, 150 ml of water was added, whereupon there was a separation of crystals. The crystalline product was collected and dried: 12.6 g of crude **12**, m.p. 156–157° ([38]: 156°).

2-Nitro-4-methoxy-5-aminotoluene (**13**). Crude **12** (16.2 g) was hydrolysed one h at 80–85° with 40% NaOH-solution (80 ml). Then 300 ml of water was added, the crystals separated were collected and dried to give 11.8 g (90%) of **13**, m.p. 130–132° ([38]: 132°).

2-Nitro-4-methoxy-5-chlorotoluene (**14**). Compound **13** (18.0 g, 100 mmol) was dissolved in a mixture of water (20 ml), glac. acetic acid (20 ml), and conc. hydrochloric acid (40 ml). A solution of sodium nitrite (7.0 g in 20 ml of water) was added dropwise with stirring, while maintaining the temperature at 0–5°, then stirring was continued for another 0.5 h. The reaction mixture was thereupon added dropwise to a vigorously stirred solution of cuprous chloride (30 g) in 100 ml of dil. HCl 1:1. The resulting mixture was heated 1 h at 70°. Addition of water (500 ml) caused crystal formation. The dried crystalline solid (18 g, 92%) melted at 91–92° (lit. [39] for **14**: m.p. 94–95°).

2-Amino-4-methoxy-5-chlorotoluene (**15**). Compound **14** (20.1 g, 100 mmol) was mixed with tin wire (60 g, 300 mmol), and conc. hydrochloric acid was added portionwise maintaining the tempera-

ture at 60–70°. After complete reduction, the reaction mixture was neutralized with dil. NaOH-solution, and extracted with chloroform (3 × 100 ml). The combined extracts were dried, then evaporated, to leave 13.5 g of dark-brown oily **15**. – NMR. ($CDCl_3$): 2.02 (*s*, 3H); 3.60 (br. *s*, 2H); 3.78 (*s*, 3H); 6.96 (*s*, 1H).

$C_8H_{10}ClNO$ (171.62) Calc. C 55.99 H 5.87 N 8.16% Found C 55.79 H 5.60 N 8.02%

2-Acetylamino-4-methoxy-5-chlorotoluene (**16**). Compound **15** (13.0 g, 76 mmol) was acylated with acetic anhydride (15 ml) at 60° for 0.5 h. After addition of water (200 ml) the separating crystals were collected and dried. Crystallization from benzene gave a sample with m.p. 166–167°. – NMR. (DMF-d_7): 2.10 (*s*, 3H); 2.20 (*s*, 3H) 3.85 (*s*, 3H); 7.22 (*s*, 1H); 7.54 (*s*, 1H); 9.27 (br. *s*, 1H).

$C_{10}H_{12}ClNO_2$ (213.66) Calc. C 56.21 H 5.66 N 6.55% Found C 56.44 H 5.83 N 6.95%

2-Acetylamino-4-methoxy-5-chlorobenzoic acid (**17**). To an aqueous solution of magnesium sulfate (24 g in 1000 ml) 12.8 g (60 mmol) of **16** was added and the mixture was heated to boiling. At this temperature dropwise addition of a $KMnO_4$-solution (35 g in 600 ml of water) was started, and was extended over a period of 8 h. When the addition was completed, conc. ammonia (25 ml) was poured into the mixture, the brown precipitate was filtered off and thoroughly washed with water. Filtrate and washings were evaporated to about 100 ml, and acidified with conc. hydrochloric acid, whereupon crude **17** separated (7.9 g, 53%). Crystallization from ethanol gave a pure sample with m.p. 250–252°. – IR.: 3330, 1692, 1660, 1608, 1580, 1509, 1465, 1390, 1335, 1270, 1245, 1225, 1199, 1116, 860. – NMR. (DMF-d_7): 2.20 (*s*, 3H); 3.97 (*s*, 3H); 7.95 (*s*, 1H); 8.50 (*s*, 1H); 11.4 (br. *s*, 2H).

$C_{10}H_{10}ClNO_4$ (243.65) Calc. C 49.30 H 4.14 N 5.74% Found C 49.50 H 4.25 N 5.75%

2-Nitroso-4-chloro-5-hydroxytoluene (**18**). 3-Hydroxy-4-chlorotoluene (7.0 g, 50 mmol, *Aldrich*), and sodium nitrite (4.25 g, 62 mmol) were dissolved in dil. NaOH-solution (2.1 g of NaOH in 120 ml of water). To this solution, cooled to 0–5°, dil. sulfuric acid (12 g of conc. H_2SO_4 in 30 ml of water) was added dropwise, which took one h. A pale-yellow crystalline product separated, and was collected, washed with water, and dried. Yield 5.6 g (65%), m.p. 185–189° (lit. [40] for **18**: 187–191° with dec.). – IR.: 3490, 3070, 2920, 1625, 1605, 1568, 1412, 1127, 1220, 1039, 980, 860, and 840. – NMR. (DMF-d_7): 2.22 (*d*, J=1.5, 3H); 6.48 (*d*, J=1.5, 1H); 7.93 (*s*, 1H); 14.0 (br. *s*, 1H).

2-Nitro-4-chloro-5-hydroxytoluene (**19**). Compound **18** (5.6 g, 32.8 mmol) was added portionwise to stirred 23% nitric acid (70 ml) preheated to 40°. After additional stirring for 1 h at RT., separated crystals were collected, washed with water, and recrystallized from ethanol to give 5.9 g (96%) of pure **19**, m.p. 138–141° ([41]: 143–144°). – IR.: 3360, 1615, 1566, 1510, 1483, 1386, 1349, 1308, 1180, 1100, 892, 850, 710. – NMR. ($CDCl_3$): 2.85 (*s*, 3H); 6.2 (br. *s*, 1H), 7.10 (*s*, 1H); 8.30 (*s*, 1H).

2-Nitro-4-chloro-5-methoxytoluene (**20**). To a stirred solution of **19** (3.0 g, 16 mmol) in 10% potassium hydroxide (10 ml), dimethyl sulfate (2.02 g, 16 mmol) was added dropwise, then stirring was continued for another hour at RT., after which an equal amount of alkali and dimethylsulfate as the first time were added. Stirring was extended for one more h at RT., then the reaction mixture was diluted with water, extracted with ether (3 × 100 ml), the combined ethereal extracts were dried and evaporated to give 3.1 g (97%) of crude **20**. A sample was crystallized from methanol: m.p. 118–119°. – IR.: 1603, 1574, 1505, 1457, 1335, 1275, 1110, 1048, 891. – NMR. ($CDCl_3$): 2.65 (*s*, 3H); 4.00 (*s*, 3H); 6.82 (*s*, 1H); 8.13 (*s*, 1H).

C_8H_8ClNO (201.61) Calc. C 47.66 H 3.99 N 6.95% Found C 47.67 H 3.80 N 6.94%

2-Amino-4-chloro-5-methoxytoluene (**21**). Compound **20** (4.5 g, 22.3 mmol) was dissolved in methanol (200 ml) and hydrogenated over *Raney* nickel (1.0 g) at RT. and ambient pressure. About 2 h were required for complete reduction. After removal of catalyst and solvent, a crude oil yproduct remained, that crystallized on cooling. The yield was quantitative, m.p. 93–94°. To obtain an analytical sample, the product was steam-distilled, then crystallized from light petroleum/benzene 9:1, after which m.p. rose to 93–94.5°. – IR.: 3405, 3320, 3220, 2850, 2830, 1634, 1500, 1220, 1053, 872, 860. – NMR. ($CDCl_3$): 2.13 (*s*, 3H); 3.40 (br. *s*, 2H); 3.80 (*s*, 3H); 6.67 (*s*, 2H).

$C_8H_{10}ClNO$ (171.62) Calc. C 55.99 H 5.87 N 8.16% Found C 56.15 H 6.01 N 8.40%

2-Acetylamino-4-chloro-5-methoxytoluene (**22**). Compound **21** (1.55 g, 2.65 mmol) was acylated with acetic anhydride (5.5 ml) as described for **16**. The crude product was recrystallized from benzene to give 1.5 g (85%) of **22**, m.p. 176–178°. – IR.: 3300, 1666, 1610, 1590, 1538, 1500, 1393, 1252, 1212,

1060, 888. – NMR. (DMF-d_7): 2.10 (*s*, 3H); 2.26 (*s*, 3H); 3.87 (*s*, 3H); 6.99 (*s*, 1H); 7.55 (*s*, 1H); 9.20 (br. *s*, 1H).

$C_{10}H_{12}ClNO_2$ (213.66) Calc. C 56.21 H 5.66 N 6.55% Found C 56.42 H 5.36 N 6.40%

2-Acetylamino-4-chloro-5-methoxybenzoic acid (**23**). A solution of $KMnO_4$ (10 g) in water (250 ml) was added by and by to a boiling solution of compound **22** (5.0 g, 23 mmol) and $MgSO_4$ (9.0 g, 75 mmol) in water (250 ml). Portions of $KMnO_4$-solution were always added after disappearance of the color due to the preceeding addition. After all permanganate was added, the solution was heated under reflux for 10 h, then cooled, and mixed with 10 ml of conc. ammonia. The dark brown precipitate was filtered off, washed with water, then filtrate and washings were reduced to a small volume by evaporation. Acidification with conc. hydrochloric acid caused precipitation of white crystals, which were collected and dried: 3.1 g (47%) of **23** was obtained. An analytical sample was prepared by recrystallizing the crude product from ethanol: m.p. 251–252°. – IR.: 3325, 2840, 2700–2500, 1705, 1670, 1610, 1603, 1585, 1250, 885. – NMR. (DMF-d_7): 2.17 (*s*, 3H); 3.95 (*s*, 3H); 7.68 (*s*, 1H); 8.73 (*s*, 1H); 9.1 (br. *s*, 1H); 11.1 (br. *s*, 1H).

$C_{10}H_{10}ClNO_4$ (243.65) Calc. C 49.30 H 4.14 N 5.74% Found C 49.38 H 4.17 N 5.57%

General procedure for preparation of **24–26**. 20 mmol of carboxylic acids **17**, **23**, and 5-chloroanthranilic (*Fluka*, puriss.), which served as starting compounds, were dissolved in acetic anhydride (30 ml), and the resulting solution was heated 4 h under reflux, with careful exclusion of moisture. After standing for an additional hour, the solution was evaporated to dryness. The residue was dissolved in dry benzene (10 ml), the solvent was removed by evaporation, and this treatment was repeated twice more. Finally the residual crystalline material was dissolved in 40 ml of dry benzene with gentle heating, whereupon 60 ml of warm cyclohexane was added. On cooling, the pure compounds (**24–26**) separated in crystalline form, and were collected and washed with cyclohexane. The products were immediately transferred to a vacuum desiccator and kept there until needed, because otherwise marked hydrolytic decomposition would occur.

6-Chloro-7-methoxy-2-methyl-4H-3,1-benzoxazin-4-one (**24**): yield 92%, m.p. 165–167°. – IR.: 1760, 1653, 1603, 1568, 1490, 1450, 1425, 1300, 1182, 1060, 970, 875, 810, 770, 742, 695. – NMR. ($CDCl_3$): 2.53 (*s*, 3H); 4.10 (*s*, 3H); 7.01 (*s*, 1H), 8.12 (*s*, 1H).

$C_{10}H_8ClNO_3$ (225.63) Calc. C 53.23 H 2.57 N 6.21% Found C 53.31 H 3.80 N 6.30%

7-Chloro-6-methoxy-2-methyl-4H-3,1-benzoxazin-4-one (**25**): yield 83%, m.p. 169–171° – IR.: 1760, 1660, 1609, 1570, 1490, 1452, 1420, 1388, 1342, 1255, 1050, 1020, 970, 888, 780. – NMR. ($CDCl_3$): 2.44 (*s*, 3H); 3.96 (*s*, 3H); 7.46 (*s*, 2H).

$C_{10}H_8ClNO_3$ (225.63) Calc. C 53.23 H 3.57 N 6.21% Found C 53.11 H 3.80 N 6.30%

6-Chloro-2-methyl-4H-3,1-benzoxazin-4-one (**26**): yield 86.3%, m.p. 124–126° ([42]: yield 80%, m.p. 143.5–146°).

General procedure for preparation of trisubstituted 2-acylaminobenzophenones **27–31**. A *Grignard* reagent was prepared by portionwise addition of an ethereal solution of bromobenzene or one of its *o*-, *m*-, and *p*-methoxy derivatives (80 mmol in 80 ml) to 80 g-atoms of magnesium ribbon and a small crystal of iodine. These additions were made under nitrogen, with vigorous stirring. The reaction was initiated by gentle warming at first, after which reflux was maintained by well-timed addition of the remainder of ethereal solution. After standing for 2 h, the ethereal layer was poured into a dropping funnel, and added dropwise to a slurry of the desired benzoxazinone (**24–26**, 55 mmol) in ether/benzene 40:80. During this addition the reaction mixture was cooled in ice/water, and after all *Grignard* reagent was added was left for 3 h in the ice/water bath. For further 4 h the mixture was kept at RT., then cooled to −15°, and 2M HCl (40 ml) was added dropwise with powerful stirring. After additional stirring for 1 h at 0°, and 0.5 h at RT., layers were separated, the aqueous phase was extracted with benzene (3 × 20 ml), the benzene extracts were combined, washed with dil. NaOH-solution, then with water, and dried. After evaporation of solvent a crude yellow crystalline material was left, which was recrystallized as indicated in the subsequent paragraphs.

2-Acetylamino-5-chloro-2'-methoxybenzophenone (**27**): yield 40% on recrystallization from benzene/hexane, m.p. 62–63°. – NMR. ($CDCl_3$): 2.27 (*s*, 3H); 3.77 (*s*, 3H); 6.9–7.7 (*m*, 6H); 8.7 (*d*, 1H); 11.5 (*s*, 1H).

$C_{16}H_{14}ClNO_3$ (303.74) Calc. C 63.27 H 4.64 N 4.61% Found C 63.50 H 4.52 N 4.80%

2-Acetylamino-5-chloro-3'-methoxybenzophenone (**28**): yield 51% on recrystallization from benzene/hexane, m.p. 66–67°. – NMR. ($CDCl_3$): 2.20 (*s*, 3H); 3.86 (*s*, 3H); 7.0–7.7 (*m*, 6H); 8.52 (*d*, 1H); 10.6 (*s*, 1H).

$C_{16}H_{14}ClNO_3$ (303.74) Calc. C 63.27 H 4.64 N 4.61% Found C 63.43 H 4.72 N 4.90%

2-Acetylamino-5-chloro-4'-methoxybenzophenone (**29**): yield 70% on recrystallization from ethanol/water, m.p. 147–149° ([43]: yield 17%, m.p. 148–149°).

2-Acetylamino-4-chloro-5-methoxybenzophenone (**30**): yield 84% on recrystallization from benzene/hexane, m.p. 121–123°. – NMR. ($CDCl_3$): 2.16 (*s*, 3H); 3.72 (*s*, 3H); 7.02 (*s*, 1H); 7.4–7.9 (*m*, 6H); 8.58 (*s*, 1H).

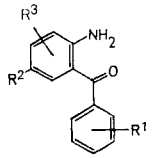
$C_{16}H_{14}ClNO_3$ (303.74) Calc. C 63.27 H 4.64 N 4.61% Found C 63.00 H 4.88 N 4.69%

2-Acetylamino-5-chloro-4-methoxybenzophenone (**31**): yield 48% on recrystallization from benzene/hexane, m.p. 162–163°. – NMR. ($CDCl_3$): 2.22 (*s*, 3H); 3.98 (*s*, 3H); 7.5 (*m*, 6H); 8.52 (*s*, 1H); 11.47 (*s*, 1H).

$C_{16}H_{14}ClNO_3$ (303.74) Calc. C 63.27 H 4.64 N 4.61% Found C 63.50 H 4.90 N 4.81%

General procedure for preparation of 2-amino-(methoxy-chloro)-benzophenones **32–36**. The desired 2-acetyl-amino-benzophenone (6.0 mmol) was dissolved in a mixture of ethanol (20 ml) and 6M HCl (15 ml). The resulting solution was first heated under reflux for 4 h, then ethanol was partly evaporated under reduced pressure. The remaining liquid was diluted with water (30 ml), made alkaline with conc. ammonia, and extracted with chloroform (3 × 30 ml). The combined extracts were washed with water, dried, and the solvent was removed by evaporation. The oily residue was dissolved in hot ethanol (10 ml), water was slowly added until appearance of turbidity, and the mixture set aside to cool and crystallize. The crystalline products were collected and characterized. The characteristic constants are given in Table 1.

Table 1. *Some physico-chemical constants of the polysubstituted benzophenones* **32–36**



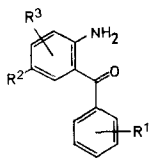
$C_{14}H_{12}ClNO_2$ (261.70) Calc. C 64.25 H 4.65 N 5.35%

Compound	R^1	R^2	R^3	m.p. [°C]	yield %	NMR[a]).	Elemental analysis Found C	H	N
32	2'-OCH_3	Cl	H	64–65	85	3.75 (*s*, 3H); 6.50 (br. *s*, 2H); 6.62 (*d*, *J*=8, 1H); 6.9–7.7 (*m*, 6H)	64.38	4.58	5.71
33	3'-OCH_3	Cl	H	73–74	80	3.83 (*s*, 3H); 6.05 (br. *s*, 2H); 6.62 (*d*, *J*=8.8, 1H); 6.9–7.7 (*m*, 6H)	64.12	4.89	5.06
34[b])	4'-OCH_3	Cl	H	97–99	86	3.87 (*s*, 3H); 5.9 (br. *s*, 2H); 6.6–7.8 (*m*, 7H)			
35	H	5-OCH_3	4-Cl	c)	88	3.86 (*s*, 3H); 5.85 (br. *s*, 2H); 6.80 (*s*, 1H); 7.02 (*s*, 1H); 7.5–8.0 (*m*, 5H)	64.57	4.50	5.17
36	H	5-Cl	4-OCH_3	143–145	80	3.90 (*s*, 3H); 6.18 (*s*, 1H); 6.2 (br. *s*, 2H); 7.4–7.6 (*m*, 6H)	64.47	4.63	5.16

[a]) Determined in $CDCl_3$.
[b]) Described in [43] (m.p. 148–149).
[c]) Yellow oil, anal. sample obtained by column chromatography.

General procedure for preparation of 2-amino-(hydroxy-chloro)-benzophenones **37–40.** Boron tribromide (7.16 g, 30 mmol) diluted with 40 ml of dry methylene chloride was added dropwise, with stirring, to a methylene chloride solution of the desired benzophenone (**32–35**, 9.0 mmol in 25 ml of the dry solvent). The addition was extended over 4 h at RT., after which stirring was continued for another 4 h. 40 ml of water was then added dropwise, to give a suspension which was extracted with ether (3 × 70 ml). The ethereal extracts were washed with 2M NaOH (2 × 25 ml) and set aside. The combined washings were acidified with 2M HCl to pH 2, and reextracted with ether (3 × 50 ml). All ether extracts were combined, washed with water, dried, and evaporated. Residual crystalline material was taken up into hot ethanol (10 ml), and crystallization was induced by gradual addition of 5 ml of hot water and subsequent chilling on ice. The products were collected and characterized. Characteristic constants are given in Table 2.

Table 2. *Some physico-chemical constants of the polysubstituted benzophenones* **37–42**



$C_{13}H_{10}ClNO_2$ (247.67) Calc. C 63.04 H 4.06 N 5.65%

Compound	R^1	R^2	R^3	m.p. [°C]	yield %	NMR.[a])	Elemental analysis Found C	H	N
37	2′-OH	Cl	H	74–76	88	6.40 (br. *s*, 2H); 6.8–7.7 (*m*, 7H); 10.6 (*s*, 1H)	63.20	4.11	5.55
38	3′-OH	Cl	H	190–192	84	6.7–7.5 (*m*, 9H, Ar+NH_2); 8.9 (*s*, 1H)	62.83	4.21	5.38
39[b])	4′-OH	Cl	H	166–168	80	6.5 (br. *s*, 2H); 6.8–7.8 (*m*, 7H); 9.83 (*s*, 1H)			
40	H	5-OH	4-Cl	165–167	86	4.9 (br. *s*, Ar−OH+NH_2+ solvent−OH); 7.12 (*s*, 1H); 7.17 (*s*, 1H); 7.6–7.8 (*m*, 5H)	63.24	4.23	5.40
41	H	5-Cl	4-OH	151–153	75	3.72 (br. *s*, 3H); 6.81 (*s*, 1H); 7.02 (*s*, 1H); 7.3–7.8 (*m*, 5H)	62.97	4.11	5.73
42[c])	H	5-Cl	3-OH	166–167	82				

[a]) Determined in acetone-d_6 (except **40** in MeOH-d_4).
[b]) Preparation described in [47], but no data presented there.
[c]) Described in [44], m.p. 166–168.

2-Amino-4-hydroxy-5-chlorobenzophenone (**41**). To an ice-cooled solution of **36** (1.57 g, 6.0 mmol) in 57% aqueous hydrogen iodide (22 ml), acetic anhydride (40 ml) was added dropwise with stirring. The resulting mixture was heated under reflux for 12 h, then diluted with 50 ml of water, and enough crystalline $Na_2S_2O_3$ was added to discharge any color due to free iodine. Extraction with ether followed (3 × 50 ml), and the extracts were washed with water, then with 2M NaOH (2 × 25 ml). The alkaline washings were acidified to pH 2–3 with 2M HCl, and reextracted with ether (3 × 50 ml). All ethereal extracts were combined, dried, and evaporated. Residual oil was purified by chromatography (60 g silica gel column, eluant benzene/acetone 100:10). Chromatographically pure material (TLC.: Rf = 0.25, solvent system benzene/acetone 100:10) was collected and crystallized from ethanol/water to obtain 1.0 g of pure **41**, m.p. 151–153° (for other data see Table 1).

General procedure for preparation of compounds **43–50**. Starting compounds – **34** and **37–42**, or 2-amino-benzophenone to prepare **50** – (5–10 mmol), were dissolved in an appropriate solvent or solvent mixture. (Solvent labels used thereafter: A = chloroform; B = methylene chloride/acetone 1:1; C = chloroform/dioxane 3:1). The solution of starting compound was supplied with a 10% molecular excess of dicyclohexylcarbodiimide (DCC), and cooled in an ice/water bath while a solution of (*S*)-[N-Cbz-alanine] (10% molecular excess) in the same solvent (10 ml per g) as the amine-DCC mixture was added dropwise with stirring. The mixture was stirred an additional 4 h at RT., then stored in a refrigerator overnight. Precipitated dicyclohexyl-urea was removed by filtration, and the residual liquid was evaporated. Products were isolated and purified by column chromatography. Appropriate eluants were sought previously by trials using TLC.

Compound **43**: from **37**, solvent A, eluant chloroform/ether 95:5. Yield 88%, m.p. 56–58° (after crystallization from ether/light petroleum 3:1). $[\alpha]_D^{25} = -41.2°$ ($c = 1.67$, $CHCl_3$). – NMR. ($CDCl_3$): 1.36 (*d*, 3H); 4.29 (*q*, 1H); 5.05 (*s*, 2H); 5.36 (*d*, 1H); 6.7–7.6 (*m*, 1H); 9.85 (*s*, 1H); 11.46 (*s*, 1H).

$C_{24}H_{21}ClN_2O_5$ (452.90) Calc. C 63.66 H 4.67 N 6.18% Found C 63.68 H 4.55 N 6.00%

Compound **44**: from **38**, solvent B, eluant chloroform/acetone 94:6. Yield 66%, m.p. 86–88° (after cryst. from diisopropyl ether). $[\alpha]_D^{23} = -20.2°$ ($c = 0.90$, $CHCl_3$). – NMR.($CDCl_3$): 1.42 (*d*, 3H); 4.38 (*q*, 1H); 5.08 (*s*, 2H); 5.72 (*d*, 1H); 6.9–7.6 (*m*, 1H); 8.41 (*d*, 1H), 11.03 (*s*, 1H).

$C_{24}N_{21}ClN_2O_5$ (452.90) Calc. C 63.66 H 4.67 N 6.18% Found C 63.39 H 4.51 N 5.92%

Compound **45**: *from* **39**, solvent C, eluant chloroform/acetone 90:10. Yield 64%, m.p. 158–160° (after cryst. from ether). $[\alpha]_D^{24} = -46.5°$ ($c = 1.22$, $CHCl_3$).

$C_{24}H_{21}ClN_2O_5$ (452.90) Calc. C 63.66 H 4.67 N 6.18% Found C 64.02 H 4.93 N 6.41%

Compound **46**: from **34**, solvent A, eluant chloroform/ethanol 99:1. Yield (crude product) 94.5%. Crystallization from ether/methylene chloride: m.p. 119–121°. $[\alpha]_D^{23} = -14.2°$ ($c = 2.4$, $CHCl_3$). – NMR. ($CDCl_3$): 1.47 (*d*, $J = 7.0$, 3H); 3.89 (*s*, 3H); 4.38 (*q*, $J = 7.0$, 1H); 5.10 (*s*, 2H); 5.63 (*d*, 1H); 6.9–7.7 (*m*, 11H); 8.57 (*d*, 1H); 10.57 (*s*, 1H).

$C_{25}H_{23}ClN_2O_5$ (466.92) Calc. C 64.32 H 4.96 N 6.00% Found C 64.14 H 4.78 N 5.81%

Compound **47**: from **40**, solvent B, eluant chloroform/acetone 90:10. Yield 68%, m.p. 163–165° (after recrystallization from ether). $[\alpha]_D = -28.6°$ ($c = 1.40$, $CHCl_3$).

$C_{24}H_{21}ClN_2O_5$ (452.90) Calc. C 63.66 H 4.67 N 6.18% Found C 63.89 H 4.49 N 5.85%

Compound **48**: from **41**, solvent C, yield (crude) 83.8%. A sample was crystallized from ether/chloroform: m.p. 220–221°. $[\alpha]_D^{23} = -38.4°$ ($c = 0.92$, methanol). – NMR. (DMF-d_7): 1.52 (*d*, $J = 7.8$, 3H); 4.26 (*q*, $J = 7.8$, 1H); 5.09 (*s*, 2H); 6.35 and 6.68 (2*d*, $J = 2.4$, 1H); 7.1–8.0 (*m*, 11H); 8.30 (*d*, $J = 2.4$, 1H).

$C_{24}H_{21}ClN_2O_5$ (452.90) Calc. C 63.66 H 4.67 N 6.18% Found C 63.79 H 4.90 N 6.19%

Compound **49**: from **42** (prepared by a *Fries* rearrangement following procedure [44]), solvent B, eluant chloroform/ethanol 99:1. Yield 79%, m.p. 149–151° (after crystallization from ether). – NMR. ($CDCl_3$): 1.43 (*d*, $J = 7.2$, 3H); 4.44 (*q*, $J = 7.2$, 1H); 5.00 (*s*, 2H); 5.66 (*d*, 1H); 6.9–7.9 (*m*, 12H); 9.7 (*s*, 1H); 11.1 (*s*, 1H).

$C_{24}H_{21}ClN_2O_5$ (452.90) Calc. C 63.66 H 4.67 N 6.18% Found C 63.44 H 4.97 N 5.95%

Compound **50**: from 2-amino-benzophenone (*Fluka*, puriss.), solvent A, yield (crude) 84.4%. A sample was crystallized from hexane: m.p. 95–97°, $[\alpha]_D^{23} = -17.5$ ($c = 1.2$, $CHCl_3$).

$C_{24}H_{22}ClN_2O_5$ (402.44) Calc. C 71.62 H 5.51 N 6.96% Found C 71.84 H 5.76 N 6.76%

7-Chloro-3-hydroxymethyl-5-phenyl-2H-1,3-dihydro-1,4-benzodiazepin-2-one (**51**). 3-Carbethoxy-5-phenyl-7-chloro-1,4-benzodiazepine (12.0 g: prepared according to [45], repeatedly crystallized from ethanol, until m.p. was 224–226°) was dissolved in dioxane (250 ml, distilled over Na). The atmosphere over this solution was replaced by nitrogen and, after cooling to 0°, dropwise of 35.0 ml of 'OMH-1' [an about 1 M solution of $LiAlH_2(C_2H_5)_2$ supplied by *Ethyl Corp.*, Baton Rouge, Indiana] was started with stirring. The addition took about 45 min, after which stirring was continued for another 30 min. Cooling was resumed, and 50 ml of 15% aqueous NaOH-solution was added drop-

wise at such a rate as to keep the mixture below +3°. When this addition was completed, pH was adjusted to 5.0 by means of dil. hydrochloric acid 1:1, and the mixture was stirred one more h while being further cooled in the ice bath. A brine (500 ml) was finally added, and the resulting slurry was extracted with ethyl acetate (3 × 200 ml). The combined extracts were dried and evaporated, and the residual oil was crystallized from ethyl acetate. The yield was 71%, and the product melted at 198–200°. – NMR. ($CDCl_3$): 3.1 (br. *s*, 1H); 3.75 (*d*, 1H); 4.3 (*m*, 2H); 6.9–7.7 (*m*, 8H); 10.0 (br., *s*, 1H).

$C_{16}H_{13}ClN_2O_2$ (300.74) Calc. C 63.91 H 4.35 N 9.31% Found C 64.00 H 4.56 N 9.66%

7-Chloro-3-camphoyloxymethyl-5-phenyl-2H-1,3-dihydro-1,4-benzodiazepin-2-one (**52**). Camphoyl chloride (1.51 g, 7.0 mmol; prepared according to [46]) was dissolved in dry pyridine (10 ml), and compound **1** (1.87 g, 6.25 mmol) was gradually added. The mixture was stirred overnight at RT., then ice/water (100 ml) was added, followed by extraction with methylene chloride (3 × 50 ml). The combined extracts were dried, evaporated, and the residual oil was purified by chromatography (50-g silica gel column, eluant: ether). The main fractions contained 2.46 g of a mixture of diastereomers of **52** which, after crystallization from methanol/water, melted at 114–117°. – NMR. ($CDCl_3$): 0.8–1.2 (*m*, 9H–CH_3); 1.6–2.7 (*m*, 5H); 3.8 (*m*, 1H); 5.0 (*m*, 2H); 7.1–7.7 (*m*, 8H); 9.8 (br. *s*, 1H).

$C_{26}H_{25}ClN_2O_5$ (480.93) Calc. C 65.05 H 5.25 N 5.84% Found C 64.92 H 5.40 N 5.60%

General procedure for preparation of compounds **53–61**. Carbobenzoxy groups of compounds **43–49** were removed by hydrolysis with HBr, that of compound **50** was removed by hydrogenolysis. With compounds where the chlorine occupies a 5-position, hydrogenolysis of the carbobenzoxy group is unsatisfactory, because it was always accompanied by partial dehydrohalogenation.

Compounds **43–49** (3–5 mmol) were dissolved in 10–15 ml of 48% HBr-solution in acetic acid by vigorous agitation. When dissolution was complete, and no further gas evolution was visible (usually after 10–15 min shaking), 20–30 ml of light petroleum was added, which caused separation of oily phases. After removal of the upper layer, oily residues were treated twice more with light petroleum, and finally dissolved in ice/water (30–50 ml). By careful addition of a saturated $NaHCO_3$-solution, the pH was adjusted to 7–7.5, and the resulting slurry was extracted with ethyl acetate (3 × 30 ml). The extracts were dried and evaporated. Purification of residues is given in descriptions of the individual compounds.

To achieve complete cyclization when preparing compounds **56**, **58**, and **59**, the residual solid from ethyl acetate extracts was redissolved in DMF (20 ml), a few drops of triethylamine were added, and the progress of cyclization was followed by taking small samples from time to time, and subjecting these samples to TLC (developing system: chloroform/acetone 90:10).

Compound **53**. Purification by column chromatography, eluant chloroform/acetone 95:5, followed by crystallization from ether. Yield 64%, m.p. 192–196°. $[\alpha]_D^{23} = +425.7°$ ($c = 1.75$, $CHCl_3$). – NMR. ($CDCl_3$): 1.73 (*d*, $J = 7.0$, 3H); 3.87 (*q*, $J = 7.0$, 1H); 6.8–7.7 (*m*, 7H); 9.3 (*s*, 1H); 13.5 (br., *s*, 1H).

$C_{16}H_{13}ClN_2O_2$ (300.74) Calc. C 63.91 H 4.35 N 9.31% Found C 63.68 H 4.56 N 9.50%

Compound **54**. Purification by crystallization from diisopropyl ether, m.p. 156–158°. – IR.: 3240, 1690, 1600, 1584, 1482, 1450, 1380, 1324, 1260–1220 (br.), 1102, 798, 702. – NMR. ($CDCl_3$): 1.72 (*d*, $J = 6.2$, 3H); 3.74 (*q*, $J = 6.2$, 1H); 3.9–4.6 (br. *s*, 1H); 7.1–7.6 (*m*, 7H); 10.07 (*s*, 1H).

$C_{16}H_{13}ClN_2O_2$ (300.74) Calc. C 63.91 H 4.35 N 9.31% Found C 63.86 H 4.50 N 9.46%

Compound **55**. In deviation from the general procedure, the oily product remaining after treatment with light petroleum was not dissolved in water and brought to neutrality. Instead, the oily material was crystallized from diisopropyl ether to give a 74% yield of crude product, m.p. 130–134°. Recrystallization from diisopropyl ether/light petroleum gave pure **55**, m.p. 136–138°. – IR.: 3240, 3130, 2940, 1772, 1695, 1611, 1584, 1482, 1440, 1373, 1210, 1197, 880, 831, 808, 700. – NMR. ($CDCl_3$): 1.70 (*d*, $J = 6.2$, 3H); 2.26 (*s*, 3H); 3.73 (*q*, $J = 6.2$, 1H); 7.0–7.7 (*m*, 7H); 9.72 (*s*, 1H).

$C_{18}H_{15}ClN_2O_3$ (342.78) Calc. C 63.07 H 4.42 N 8.17% Found C 63.24 H 4.44 N 8.30%

Compound **56**. Cyclization in DMF required 48 h at RT.. Solvent evaporation at 0.01 Torr. The residual oil was crystallized from methanol, m.p. 296–298°. $[\alpha]_D^{23} = +196°$ ($c = 1.02$, acetone). –

NMR. (DMF-d_7): 1.60 (*d*, J=7.2, 3H); 3.71 (*q*, J=7.2, 1H); 3.5–4.5 (br. *s*, 1H); 6.8–7.6 (*m*, 7H); 10.2–10.6 (br. *s*, 1H).

$C_{16}H_{13}ClN_2O_2$ (300.74) Calc. C 63.91 H 4.75 N 9.32% Found C 63.89 H 4.37 N 9.20%

Compound **57**. Purification by column chromatography, eluant methylene chloride, followed by crystallization from ether. M.p. 110–112°, $[\alpha]_D^{23} = +148°$ (c=1.12, $CHCl_3$). – NMR. ($CDCl_3$): 1.73 (*d*, J=7.0, 3H); 3.73 (*q*, J=7.0, 1H); 3.80 (*s*, 3H); 6.8–7.8 (*m*, 7H); 10.0 (*s*, 1H).

$C_{17}H_{15}ClN_2O_2$ (314.77) Calc. C 64.87 H 4.80 N 8.90% Found C 64.93 H 4.71 N 8.71%

Compound **58**. Cyclization in DMF required 2 h at RT. Residual oil remaining after solvent evaporation was crystallized from ether, m.p. 253–258°. Purification by column chromatography, eluant chloroform/acetone 90:10, followed by crystallization from acetone. M.p. 255–258°, $[\alpha]_D^{23} = +311°$ (c=0.82, acetone).

$C_{16}H_{13}ClN_2O_2$ (300.74) Calc. C 63.91 H 4.35 N 9.31% Found C 63.66 H 4.57 N 9.09%

Compound **59**. Cyclisation in DMF required 24 h at RT. Purification by column chromatography, eluant chloroform/methanol 94:6, followed by crystallization from chloroform/light petroleum. M.p. 294–296°. – NMR. (acetone-d_6): 1.60 (*d*, J=7.2, 3H); 4.76 (*q*, J=7.2, 1H); 6.6–7.7 (*m*, 8H); 9.45 (*s*, 1H).

$C_{16}H_{13}ClN_2O_2$ (300.74) Calc. C 63.91 H 4.35 N 9.31% Found C 64.31 H 4.34 N 9.20%

Compound **60**. Purification by column chromatography, eluant ether, followed by crystallization from acetone/water. M.p. 175–178°, $[\alpha]_D^{23} = +214°$ (c=1.10, methanol). – NMR. (methanol-d_4): 1.63 (*d*, J=7.2, 3H); 3.66 (*q*, J=7.2, 1H); 4.78 (*s*, 1H); 6.65 (*d*, J=2.4, 1H); 7.04 (*d*, J=2.4, 1H); 7.43 (*s*, 5H); 7.65 (br. *s*, 1H).

$C_{16}H_{13}ClN_2O_2$ (300.74) Calc. C 63.91 H 4.35 N 9.31% Found C 64.12 H 4.65 N 9.04%

Compound **61**. 10 g (25 mmol) of **50** was dissolved in 150 ml of 96% ethanol and hydrogenolysed at RT. in the presence of 1.0 g of 10% Pd/C. Completeness of the reaction was indicated after 6 h by TLC. (chloroform/acetone 90:10). After removal of the catalyst by filtration, the solution was stirred overnight and, at the end of this period, only pure **61** was detected by TLC. Evaporation of solvent left an oily residue which, after crystallization from acetone/water, yielded 4.9 g (78%) of the pure product, m.p. 160–162°. – NMR. ($CDCl_3$): 1.75 (*d*, J=6.6, 3H); 3.75 (*q*, J=6.6, 1H); 6.9–7.7 (*m*, 9H); 10.0 (br. *s*, 1H).

$C_{16}H_{14}N_2O$ (250.31) Calc. C 76.77 H 5.63 N 11.20% Found C 76.98 H 5.75 N 11.30%

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