These results show that the tricyclic antidepressants reduced the effects of PGE_2 in the guinea pig ileum and in the mouse central nervous system. It was not possible to quantify the antagonism in the mouse because recovery was hardly achieved. This study indicates that interactions of PGE_2 and the tricyclics in the ileum is similar to the interactions of the tricyclics and 5-hydroxytryptamine (5-HT) in the same preparation[6]. In addition, methysergide also reduced the effects of PGE_2. Reduction of the contractile action of PGE_2 in reserpinized ileum may indicate that some of its actions are mediated via the release of a spasmogen. Since both antidepressants are also potent non-competitive antagonists of 5-HT[6], it can be speculated that some of the actions of PGE_2 may be exerted via 5-HT. The monoamine theory of depression[7] highlights the significance of 5-HT in depression. Tricyclics, more specifically, amitriptyline and clomipramine[8] are more potent inhibitors of 5-HT than they are of noradrenaline uptake. In conclusion, it would appear that PGE_2 produces contractions of the guinea-pig ileum and hypomotility in the mouse via a sertonin-like mechanism.

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Indoxyl derivatives of drug metabolites

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Summary. Indoxyl derivatives were detected as minor products among the urinary metabolites of two trial drugs, a benzodiazepine (GP 55 129) and a benzophenone (CGP 11 952). Their structures were elucidated by NMR and mass spectroscopy. Presumably, metabolites containing potential aldehyde functions react spontaneously with endogenous indoxyl. Such derivatives have not hitherto been encountered in drug metabolism.
Key words. Drug metabolism; indoxyl derivatives; aldehyde intermediates; benzodiazepines; benzophenones.

Indoxyl (**I**) is a product of tryptophan degradation in the mammalian organism[3]. In urine it is excreted in considerable amounts as O-sulphate (**II**) and O-glucuronide (**III**)[4,5] (fig. 1). Healthy adult persons, for instance, excrete about 60–200 mg of **II** per day[6]. In the rat the amount is about 3 mg per day[7]. Recently we investigated the biotransformation of two trial drugs in rat and dog. GP 55 129 (**IV**)[8] is a benzodiazepine, and CGP 11 952 (**V**)[9] is a benzophenone (fig. 2). Both display central nervous activities. The two trial drugs were administered in ^{14}C-labeled form. Among the numerous radioactive compounds found in urine, some contained an indoxyl moiety as part of their structure.

Indoxyl derivatives of drug metabolites have not been described so far. In the present paper we therefore wish to report on the isolation and structure elucidation of these derivatives.

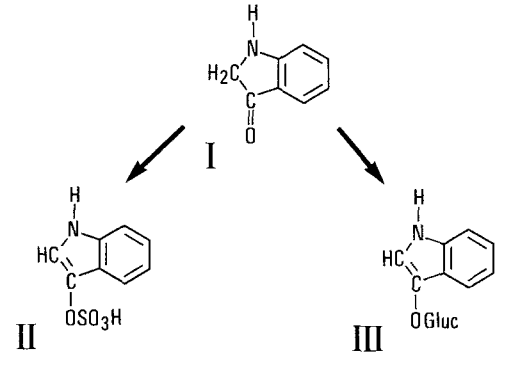

Figure 1. The structures of indoxyl (**I**), and of indoxyl conjugates excreted in urine (**II**, **III**) (Gluc = glucuronic acid residue).

Materials and methods. ^{14}C-Labeled GP 55 129[8] and CGP 11 952[9] had specific radioactivities of 18 and 23 kBq/mg, respectively. The synthetic, non-labeled indoxyl derivatives CGP 27 729 (**VI**)[10] and H.A. 2800.2.1 (**VII**)[11] were available as reference substances. Solvents and chemicals were of reagent grade and were obtained from Fluka (Buchs, Switzerland) or E. Merck (Darmstadt, FRG).

Rats (N = 12) received oral doses of 50 mg/kg of [^{14}C] GP 55 129 on two consecutive days. Dogs (N = 5) received oral doses of 5 mg/kg of [^{14}C] CGP 11 952 singly, or on two conse-

Spectroscopic data of **VI** and **VIII**

VI	NMR (CD_3OD):	7.93, 7.92 and 7,68 (H-3, H-5 and H-6 of the 4-chlorophenyl ring); 7.62, 7.56, 7.11 and 6.98 (H-4, H-6, H-7 and H-5 of the indolinone moiety); 7.32, 7.29,7.18 and 7.16 (H-6, H-4, H-3 and H-5 of the o-chlorophenyl ring); 6.14 (s, vinyl proton).
	MS m/e (%)*:	503(100) M(2 Cl); 468 (3) M-Cl; 423 (2) 468-NH_3/CO; 364(20) M-C_6H_4ClCO; 347(10) 364-NH_3; 319(6) 347-CO; 139(34) C_6H_4ClCO; 111(15) 139-CO.
VIII	NMR (CD_3OD):	7.83, 7.71 and 7.63 (H-5, H-3, and H-6 of the 4-chlorophenyl ring); 7.58 and 6.70 (AA' BB' system, p-hydroxyphenyl ring); 7.55, 7.49, 7.08 and 6.92 (H-4, H-6, H-7 and H-5 of the indolinone moiety); 5.82 (s, vinyl proton); 4.66 and 4.53 (AB system, J = 14 Hz, CH_2).
	MS m/e (%)*:	472(100) M($C_{25}H_{17}ClN_4O_4$). 351(13) M-C_6H_4OHCO.
	IR (KBr):	1705, 1655, 1631 and 1608 cm^{-1}.
	UV (CH_3OH):	λ_{max} = 279, 300, 479.

* Peaks due to ^{37}Cl are neglected.

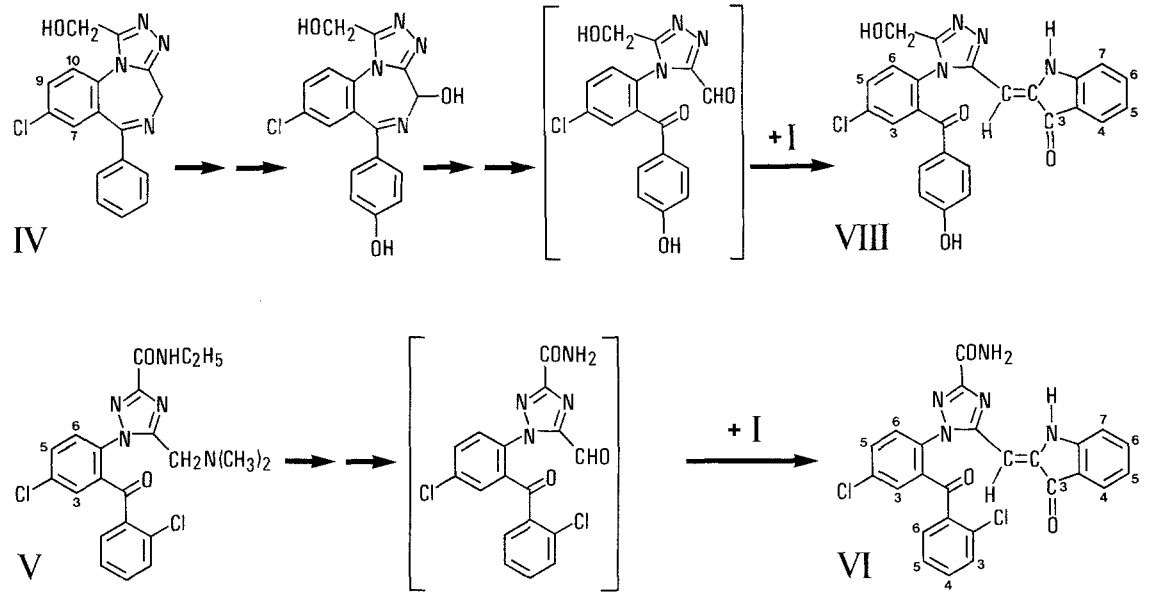

Figure 2. Proposed pathways for the formation of indoxyl derivatives. Upper part: Transformation of **IV** (GP 55 129) to **VIII**. Lower part: Transformation of V (CGP 11 952) to **VI**.

cutive days. Urine was collected for up to 96 h. The animals had been raised on the premises of Ciba-Geigy (male albino rats Tif: RAIf [SPF]; male pedigreed beagle dogs).

The ^{14}C-compounds contained in rat urine were adsorbed on Amberlite XAD-2 (35–50 mesh) and eluted with a linear gradient of ethanol in water. The concentrate obtained was partitioned between water and ethyl acetate at pH 8. The metabolites in the organic phase were separated by preparative LC on Merck LiChrosorb Si 60, 10 µm (column size 300 × 25 or 300 × 12.5 mm I.D.). The solvent mixtures used for elution contained components of low, medium and high polarity, e.g. cyclohexane-ethanol-conc. aq. ammonia (70:30:1, by vol.). Metabolites were traced and quantitated by radiodetection. After three consecutive runs, **VIII** was obtained in pure form. The same separation principles applied to dog urine yielded compound **VI**. Technical details of instruments and methods are described elsewhere[12].

The structures of **VI** and **VIII** were elucidated by NMR and mass spectroscopy. ^{1}H- and ^{13}C-NMR spectra were measured in CD_3OD solution with a Bruker HX 360, the IR spectra with a Perkin-Elmer 580 B, and the UV spectra with a Cary 118 instrument. Low resolution mass spectra were obtained with a Varian MAT CH5-DF mass spectrometer (direct sample insertion, 70 eV ionizing energy, 180 °C ion source temperature), attached to a SS-100 Data System. A CEC 21-110 B instrument provided the high resolution data (photographic detection on Ionomet plates).

For quantitative measurement of **VI** in urine, reverse isotope dilution analysis was used. Non-labeled **VI**[10] (30 µg) was added to the sample (2–6 ml). CGP 11 952 and 10 synthetic metabolites were also added (10 µg each), to ensure specificity of the assay. After extraction, **VI** was isolated from the mixture by repeated LC. The ultimate run was made on Merck LiChrosorb Si 60, 10 µm (250 × 4.6 mm ID) with 1,2-dichloro-ethane-2-propanol-conc. aq. ammonia (957:40:3). The amount of **VI** in the urine sample was determined by on-line UV-measurement at 280 nm, and off-line radiometry of the peak fraction.

Results and discussion. Compound **VIII** results from one of the minor pathways of the biotransformation of GP 55 129 (**IV**) in the rat (fig. 2). The amount of **VIII** detected in urine was of the order of 1% of the ^{14}C-dose. The main pathway of **IV** in this species involves hydroxylation at C-4 of the mono-substituted phenyl ring, followed by glucuronidation[13].

Compound **VI** is formed in similarly small amounts as a product of biotransformation of CGP 11 952 (**V**) in the dog (fig. 2). Here, the main metabolic reactions proceed through ring closure of **V** to benzodiazepine structures[14], as is also known for other drugs of the benzophenone series[15].

The structures of the products isolated from urine (**VI**, **VIII**) were deduced from the spectroscopic data given in the table. These structures were confirmed by spectroscopic comparison with synthetic **VI**[10] and **VII**[11], the latter being an analogue of **VIII**. Low- and high-resolution mass spectrometry yielded the molecular weights of **VI** and **VIII**, and their elemental composition. In the NMR spectra of **VI** and **VIII**, the chemical shifts of the protons of the 4-chlorophenyl ring (7.6–7.9 ppm) indicate a benzophenone rather than a benzodiazepine structure (cf. 3-H, 5-H and 6-H in V: 7.78, 7.84 and 7.81 ppm; 7-H, 9-H and 10-H in IV: 7.40, 7.82 and 8.16 ppm). This is also obvious from the mass spectra. Upon electron impact ionization, **VI** and **VIII** lost a chlorobenzoyl and a hydroxybenzoyl radical, respectively. From **VI**, in addition, the chlorobenzoyl cation was formed, which further decomposed by loss of CO. The chemical shifts of the vinyl and aromatic protons in addition to those of the benzophenone system are characteristic for the 2-methylene-3-indolinone moiety[16,17]. The vinyl protons in **VI** and **VIII** absorb at rather high field, as compared with other 2-arylmethylene-3-indolinones[17]; they are shielded by the 4-chlorophenyl ring attached to the triazole ring systems. This is corroborated by the observation of an AB system for the CH_2O group in **VIII**, indicating that the molecule is chiral on the NMR time scale. The vinyl protons are cis to the carbonyl C-atom (C-3), as shown by the coupling constant[18] $^{3}J_{CH}$ in the synthetic reference substance **VII**[11]: The signal of C-3 in a proton coupled ^{13}C-NMR spectrum is a multiplett with $\Sigma J = 9$ Hz which represents a $^{3}J_{CH}$ of ≤ 6 Hz, after accounting for the coupling between 4-H and C-3. The Z configuration, furthermore, is in agreement with the IR and UV data and a previous study of the stereochemistry of 2-arylmethylene-3-indolinones[17].

The proposed metabolic pathway leading from GP 55 129 to the indoxylidene derivative **VIII** begins with hydroxylation at C-4 of the benzodiazepine ring system, and at C-4 of the monosubstituted phenyl ring (fig. 2). Spontaneous ring opening and deamination yield a benzophenone with an aldehyde function. Analogous reactions have been reported for other benzo-

diazepines[19,20]. The presumed pathway from CGP 11952 to **VI** begins with oxidative deamination at the dimethylaminomethyl side-chain, which again yields an aldehyde (fig. 2). This is accompanied by oxidative dealkylation at the ethyl carboxamide side-chain. Formally, the two aldehyde intermediates react with indoxyl (**I**) by loss of one molecule of water. It is probable, however, that the aldehyde intermediates are present as derivatives, e.g. as Schiff-bases with biogenic amines.

Quantitative determination of **VI** in the urine of dogs dosed with [^{14}C] CGP 11952 provided some insight into the mechanism of the above reaction. In urine samples which were stored for a few hours at room temperature, **VI** was readily detected by reverse isotope dilution analysis. In freshly voided urine, collected at 0 °C, **VI** was below the detection limit. This suggests that **VI** and **VIII** are formed only after their aldehyde precursors have passed the kidneys. The reaction seems to be spontaneous rather than enzymic. Aldehydes do indeed react with indoxyl or indoxyl conjugates. A standard method for the measurement of urinary indoxyl is based on its reactions with 4-dimethylaminobenzaldehyde[21].

Mammalian urine contains ample amounts of endogenous indoxyl[6,7]. Xenobiotics can be transformed into aldehyde intermediates by various metabolic reactions[19,20,22]. Therefore, indoxylidene derivatives as detected in this study may also be found in other cases.

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Rate of tryptophan hydroxylation in vivo in brain nuclei of genetically hypertensive rats of the Lyon strain[1]

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Summary. The rate of tryptophan hydroxylation in vivo is unaltered in brain areas of 5, 9 and 21 week-old Lyon genetically Hypertensive (LH) rats as compared to both Lyon Normotensive (LN) and Low Blood Pressure (LL) rats, except for a decrease in the C1 area of the medulla oblongata in 9 week-old animals.
Key words. Genetically hypertensive rats; serotonin synthesis; tryptophan hydroxylase.

Previous work has permitted the simultaneous selection of one strain of genetically hypertensive rats and of two control strains, which are denominated respectively Lyon Hypertensive (LH), Normotensive (LN) and Low Blood Pressure (LL) strains[2]. As there is evidence that central serotonergic neurons are involved in the control of blood pressure[3], it seemed interesting to determine whether they are functionally altered in this model (LH rats). In the present work, the rate of tryptophan hydroxylation in vivo, which can be considered as a biochemical index of the activity of serotonergic neurons[4], was estimated[5] in discrete brain nuclei of male LH, LN and LL rats. The animals were studied at three ages which characterize the evolution of the blood pressure in these strains, i.e. at 5 and 9 weeks (which correspond respectively to the beginning and to the end of the onset of the hypertension) and at 21 weeks when the hypertension is established[2].

The rate of tryptophan hydroxylation in vivo was estimated by measuring the accumulated 5-hydroxytryptophan (5-HTP) following the pharmacological blockade of the aromatic L-amino acid decarboxylase by NSD 1015[6]. The rats were sacrificed 30 min after the administration of NSD 1015 (50 mg/kg i.p.) and the brains were quickly removed. The following brain areas were dissected out[7]: a) three regions containing serotonin cell bodies: the nuclei raphe centralis, dorsalis and magnus[8], and b) four regions containing serotonin terminals: the medullary C1 and C2 adrenergic regions[9] and the anterior and posterior parts of the hypothalamus. The brain samples were homogenized and after centrifugation the supernatants were used for the 5-HTP determination which was carried out by a sensitive radioenzymatic assay[5]. The proteins were determined in the pellet[10]. As LH, LN and LL rats of the same age were simultaneously killed and assayed, they can be compared safely. No attempt has