The constituents of mixtures, such as kaolin and morphine mixture B.P.C., with high solids content are usually determined on a weight in weight basis. We were able to pipette the homogeneous mixture providing that the pipette was allowed to drain thoroughly. This is reflected in the recoveries of morphine from the mixtures, and we therefore recommend that the determination for total anhydrous morphine be on a weight in volume basis.

The 'free' morphine content (that in the dekaolinated solution) of the mixtures was determined by taking a portion of the homogeneous mixture and centrifuging it in such a way that 20·0 ml of the supernatant liquid could be pipetted into a separator. To this were added 5 ml ethanol (96% v/v), 5 ml water and 2 ml dilute ammonia solution (10% w/w). This mixture was extracted with 3 × 30 ml of a 2:1 mixture of chloroform and ethanol. Each chloroform fraction, after separation, was washed with the same 20 ml of a 1:1 mixture of ethanol and water. The method of assay was continued as previously described for the determination of the total anhydrous morphine content commencing at the words '. . . The combined chloroform fractions were evaporated . . .'.

The 'free' morphine content of the mixtures under test is given in Table 1(b). We found that only about 75% of the morphine content appears to be 'free' in solution.

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On the mechanism of metabolic *N*-dealkylation. Isolation of a relatively stable carbinolamine

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Current evidence does not indicate whether tertiary amines are dealkylated by initial α*C*-oxidation, or by initial *N*-oxidation and subsequent *N*-oxide dealkylation (Bickel 1969; Hucker 1973). In the case of clebopride (I, Table 1; Cleboril), a new benzamide drug (Prieto et al 1977; Roberts et al 1978), previous identification of in vitro metabolic products had indicated amide hydrolysis (product III), *N*-oxidation (product VI), *N*-dealkylation (product II), and *N*-dealkylation, followed by *C*-oxidation (product V) or *N*-oxidation (products VII and VIII) (Huizing & Beckett 1980; Huizing et al 1979b, 1980).

We now report that when I was incubated with 9000 *g* supernatant of liver homogenates of male NZW rabbits (Cowan et al 1976), and an extract of the incubation mixture subjected to t.l.c. (Huizing et al 1979a), metabolite IV was observed, in addition to the products mentioned above (R_F values are given in Table 1). A positive diazo-coupling reaction for IV indicated an intact aromatic amino group. Unlike the basic metabolic product III, but like the *N*-oxide VI, compound IV was extractable from incubation mixtures into chloroform, at all pH values, suggesting a neutral character for IV. Although IV is unstable at room temperature (one of the breakdown products of IV is I), compound IV may be purified by preparative t.l.c.

Treatment overnight with 2M sulphuric acid at 95 °C, decomposes IV to various unidentified products. Treatment with Zn/HCl of mixtures containing IV and VI, resulting from incubation of I with 9000 *g* supernatant of rabbit liver, gave a significant reduction of IV and VI, whereas treatment with $KMnO_4$ did not produce important changes (for reduction and oxidation procedures, see Huizing et al 1979b).

Field desorption (FD)*-mass spectrometry of IV gave an abundant molecular ion at *m/z* 389 (wire current 17 mA) (i.e. 16 atomic mass units higher than that of the parent compound I; see Huizing et al 1980), further ions were observed at *m/z* 390 (M + 1) and at *m/z* 412 (M + 23, i.e. M + Na) at a wire current of 13 mA; such ions are commonly observed in FD-mass spectrometry (Kirk et al 1976; Wilson 1977). The observed decrease in relative abundance for the ions at M + 1 and M + 23 and the initial appearance and further increase in abundance for the M^+ ion, when the wire current was increased from 13 to 17 mA, is in agreement with the rules proposed by Schulten & Beckey (1974). The mass spectrum of IV, determined under

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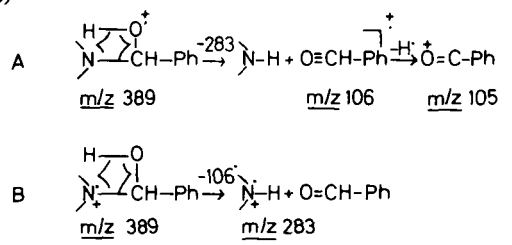
* For the FD-mass spectrometry studies a double beam AEI MS 30 and a single beam AEI MS 50 instrument were used, in the direct inlet mode; the samples were coated on a tungsten emitter wire by the dipping technique of Schulten & Beckey (1974) from solutions in methanol; the reference beam was used for mass marking, using the electron impact mass spectrum of perfluorokerosene No. 4, high boiling grade.

electron impact, was similar to that of I (Huizing et al 1980), differing only in containing significant ions at *m/z* 105 and *m/z* 106, with the ion at *m/z* 283 more abundant than that at *m/z* 282. These observations can be explained by the elimination (A) and rearrangement (B) below:



Thus, metabolic product IV is concluded to be the carbinolamine *N*-(1′-α-hydroxybenzyl-4′-piperidyl-4-amino-5-chloro-2-methoxybenzamide.

When the incubation of I with fortified 9000 *g* supernatant of liver homogenates of male NZW rabbits was terminated at different times, the relative amounts present of metabolic products III, IV and VI indicated that the *N*-dealkylated product III was formed at the expense of the intermediate carbinolamine IV. By contrast, the concentration of the tertiary *N*-oxide VI (initially detected after approx. 20 min incubation) increased at a rate similar to that of III. These results demonstrate that metabolic *N*-debenzylation proceeds via the formation of an intermediate carbinolamine, and not via *N*-oxidation.

A similar carbinolamine has been isolated, as a glucuronide, from the urine of subjects given *N*-benzyl-carbethoxy-hydropamate (Edelson et al 1968). This metabolite dehydrated on hydrolysis, forming an oxime, in which the C=N bond is conjugated with the aromatic nucleus. For IV, such a dehydration cannot take place, since the N is part of a ring system. It has also been observed that the benzaldehyde, produced by the in vitro *N*-debenzylation of *N*-benzyl-4-phenyl-4-carbethoxy-piperidine in an $^{18}O_2$ environment, contained ^{18}O (McMahon et al 1969), thus indicating the lack of involvement of the *N*-oxide in *N*-debenzylation. In a more recent study (McMahon & Culp 1979), the oxidative *N*-debenzylation of 1-benzyl-4-cyano-4-phenylpiperidine was investigated with respect both to the source of oxygen and any deuterium isotope effect. Molecular oxygen was shown to be involved, and a positive isotope effect was observed for the two methylene hydrogen atoms of the benzyl group in accordance with the proposition that the *N*-debenzylation reaction of the present study proceeds by direct hydroxylation. Thus it is a typical microsomal hydroxylation in which the primary metabolite is a carbinolamine (viz. IV) which in turn dissociates nonenzymatically to the dealkylated amine (III) and an aldehyde.

Table 1. Structures and silica gel t.l.c. R_F values of I and its metabolic products III, IV and VI.

No.	Structure	R_F in solvents 1–4			
		1	2	3	4
I	R-CH_2Ph	0·61	0·65	0·56	0·63
III	R-H	0·02	0·07	0·40	0·10
IV	R-COH(H)Ph	0·11	0·15	0·56	0·34
VI	R(O*)-CH_2Ph	0·19	0·19	0·62	0·41

R = *N*-(1′substituent-4′-piperidyl)-4-amino-5-chloro-2-methoxybenzamide; Ph = phenyl; * = oxygen atom attached to nitrogen of piperidine nucleus; solvent systems methanol–chloroform (1:4) (1), 1,2-dichloroethane–ethanol–ammonia solution sp. gr. 0·88 (70:15:2) (2), n-butanol–acetic acid–water (4:1:1) (3) and isopropanol–ammonia solution sp. gr. 0·88–water (80:4:5) (4).

Since the present work was completed, IV was detected as one of the in vivo metabolic products of clebopride (I) in rats, rabbits, dogs and man (Segura et al 1980).

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