1-Piperidinocyclohexanecarbonitrile, a toxic precursor of phencyclidine

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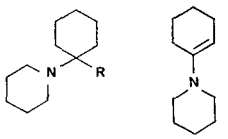
In the course of preparing analogues of phencyclidine (I) for identification purposes (Bailey, Gagné & Pike, 1976), we characterized 1-piperidinocyclohexanecarbonitrile(II) which is an intermediate in phencyclidine synthesis (Kalir, Edery & others, 1969). This synthetic route for (I) and its analogues is employed in illicit laboratories which usually monitor the quality of their products in the most rudimentary fashion. We observed that if the step involving reaction of (II) with a Grignard reagent did not proceed, subsequent work-up designed to yield the hydrochloride of phencyclidine or its analogues produced crystalline 1-piperidinocyclohexanecarbonitrile hydrochloride (IIa). Reed & Kane (1970) reported that a missynthesis can possibly produce a toxic substance, and we surmised that it might be (IIa) which is an organic cyanide. We wish to report our identification of (II) in police seizures, and the results of our preliminary investigation into its toxicity.



I, R=C_6H_5 III

II R=CN

The seized crystalline material (m.p. 180–182°) dissolved in water and the solution gave a positive test for chloride ion on adding silver nitrate solution. The infrared spectrum showed strong absorption bands at 2200–2600 cm^{-1}, assigned to R_3N^+H, suggesting that the substance could be the hydrochloride of a tertiary amine. The absence of strong infrared absorption bands from 700–850 cm^{-1} indicated that no aromatic substituent was present. The base was generated by dissolving the solid in sodium carbonate solution, followed by extraction with ether. Its infrared spectrum showed absorption at 2220 cm^{-1}, suggesting that a cyanide or, less probably, an acetylene moiety was present. The salt and the base were examined by proton magnetic resonance (pmr) spectroscopy: only saturated aliphatic protons were detected. At this point, structure (II) was surmised for the unknown and comparison of infrared, pmr, mass spectra and thin-layer chromatograms with those of an authentic sample confirmed the identification. Detailed methods for the identification of (II) and its analogues will be published (Pike, Gagné & Bailey, in preparation).

It seemed likely that the alleged toxicity of impure phencyclidine could be ascribed to the presence of the nitrile (II), which could possibly generate toxic hydrogen cyanide and produce 1-cyclohexenylpiperidine (III) following ingestion. The alcoholic solutions of (II) on heating with alcoholic silver nitrate, slowly gave a faint turbidity which could be due to the formation of silver cyanide and the gas-liquid chromatograms of (II) and of (III) had the same retention times (Pike, Gagné & Bailey, in preparation). Pmr spectroscopy indicated that solutions of (II) in $CDCl_3$ were stable at 38°, but that heating (IIa) in D_2O in an attempt to obtain a concentrated solution resulted in its decomposition into cyclohexanone and piperidine (the presumed intermediate (III) was not detected). The decomposition of (IIa) in D_2O at 38° was monitored by pmr spectroscopy and found to follow first order kinetics ($k_1 = 0{\cdot}39\ h^{-1}$).

Authentic (I), (II), (IIa) and (III) were synthesized and their toxic effects in male Swiss-Webster mice, 26–36 g, were investigated. Mice had free access to food and water at all times. Compounds (I) and (IIa) were dissolved in distilled water whereas (II) and (III) were dissolved in corn oil. All solutions were freshly prepared and used immediately. Unless otherwise indicated, dosing solutions were administered to mice at 10 ml kg^{-1}.

The 24 h oral median lethal dose (LD50) for (I), (II), (IIa) and (III) was determined by logit analysis (Part I, Waud, 1972) and the 95% confidence limits were obtained by the application of equation 4·35 from Finney (1971). Results presented in Table 1 suggests that (IIa) is marginally more toxic than (I) but that (II), dissolved in corn oil, is 2 to 3 times less toxic than the corresponding hydrochloride salt (IIa), which may be due to differences in absorption through the gut. Since 1-cyclohexenylpiperidine (III), a possible metabolite of (II) and (IIa), is relatively non-toxic (LD50 = 1270 mg kg^{-1}) the possibility of cyanide toxicity following oral administration to mice of (II) and (IIa) was investigated.

Way, Gibbon & Sheehy (1966) showed that the LD50 of potassium cyanide (0·13 mmol kg^{-1}) in mice can be increased 2·5-fold by pretreatment with sodium nitrite alone, 4-fold by sodium thiosulphate alone and 6·3-fold

Table 1. *Oral lethal doses in mice (24 h)**.

Substance		LD50 (mg kg^{-1})	LD50 (mmol kg^{-1})
Phencyclidine HCl (I)		76·5 (63·2–87·3)	0·274 (0·226–0·312)
1-Piperidino-cyclohexane-carbonitrile	base II	133·0 (119·3–152·0)	0·693 (0·621–0·792)
	HCl IIa	59·5 (54·9–64·5)	0·260 (0·240–0·282)
1-Cyclohexenyl piperidine (III)		1272·7 (1065·6–1456·5)	7·713 (6·458–8·827)

* Groups of 10 mice were used to determine the LD50. The 95% confidence limits for LD50 are given in parentheses.

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by a combination of both antidotes. This forms the basis of the treatment of cyanide toxicity and the mechanisms of the antidotal action are that the treatment with nitrite forms methaemoglobin and so complexes with the cyanide, and the thiosulphate accelerates the metabolic conversion of cyanide into thiocyanate. Using an experimental approach similar to that of Way, Gibbon & Sheehy (1966), the protective actions of sodium nitrite and sodium thiosulphate on the toxicity of (II) and (IIa) were studied. Results are given in Table 2. Sodium nitrite and sodium thiosulphate individually afforded some protection against the lethal effects of (IIa). However, pretreatment of mice with both sodium nitrite and sodium thiosulphate afforded increased protection against the lethal effects of (II) and (IIa). Sodium nitrite and sodium thiosulphate did not protect against lethality produced by (I). On the basis of these results we speculate that CN^- may be a metabolite of (II) and (IIa) in mice.

The potential of (IIa) to produce hepatotoxic and nephrotoxic effects was also assessed. Groups of 10 mice were dosed with vehicle (H_2O) or (IIa) at 45, 50, and 55 mg kg^{-1}, and 24 h later, blood obtained in heparinized syringes by cardiac puncture. As an index of hepatotoxicity, plasma concentrations of glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) were monitored. Blood urea nitrogen (BUN) was used as an indicator of nephrotoxicity. Our results show that concentrations of GOT, GPT and BUN in mice treated with (IIa) did not differ from those in control mice, indicating that (IIa) is probably not hepatotoxic or nephrotoxic.

Table 2. *Effects of sodium nitrite and sodium thiosulphate on the toxicity of 1-piperidinocyclohexanecarbonitrile and phencyclidine.*

Pretreatment[a]	Challenge[b]	No. mice dead / No. mice used
$NaNO_2$	H_2O	0/8
$Na_2S_2O_3$	H_2O	0/8
$NaNO_2 + Na_2S_2O_3$	H_2O	0/8
H_2O	(IIa)	6/8
$NaNO_2$	(IIa)	1/8
$Na_2S_2O_3$	(IIa)	3/8
$NaNO_2 + Na_2S_2O_3$	(IIa)	0/8
H_2O	(II)	5/6
$NaNO_2 + Na_2S_2O_3$	(II)	0/6
H_2O	(I)	7/10
$NaNO_2 + Na_2S_2O_3$	(I)	8/10

[a] $NaNO_2$ (20 mg kg^{-1}) and $Na_2S_2O_3$ (200 mg kg^{-1}) were administered intraperitoneally.

[b] Mice were challenged with 70 mg kg^{-1} of (IIa) or 100 mg kg^{-1} of (I) dissolved in H_2O and 180 mg kg^{-1} of (II) dissolved in corn oil, administered by oral intubation 10 min after pretreatment with $NaNO_2$ (20 mg kg^{-1}) and /or $Na_2S_2O_3$ (200 mg kg^{-1}).

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REFERENCES

BAILEY, K., GAGNÉ, D. & PIKE, R. K. (1976). *J. Ass. off. agric. Chem.*, **59**, 81–89.
FINNEY, D. J. (1971). *Probit Analysis*, p. 78, London: Cambridge University Press.
KALIR, A., EDERY, H., PELAH, Z., BALDERMAN, D. & PORATH, G. (1969). *J. medl Chem.*, **12**, 473–477.
REED, A. & KANE, A. W. (1970). *Stash Capsules*, **2** (5), 1–2.
WAUD, D. R. (1972). *J. Pharmac. exp. Ther.*, **183**, 577–607.
WAY, J. L., GIBBON, S. L. & SHEEHY, M. (1966). *Science*, **152**, 210–211.

Variations of the enzyme inducing effects of contraceptive agents in different animal species

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Interactions of contraceptive steroids with the liver microsomal enzymes and in general with drug metabolism has been reported (Blackham & Spencer, 1969; Juchau & Fouts, 1966; Jori, Bianchetti & Prestini, 1969; Rümke & Noordhoek, 1969; Freudenthal & Amerson, 1974; Garg & Ahmad, 1974; Soyka & Deckert, 1974; Tüttenberg, Hüthwohl & others, 1974; Freudenthal, Amerson & others, 1974). However, various animal species and different experimental approaches and contraceptive steroid combinations were employed, with conflicting results.

We have compared the effect of some of the most frequently used combinations of steroid contraceptives on the liver microsomal enzyme activity in rat, mouse and guinea-pig.

We also report the results of previous experiments in which the antifertility activity of the contraceptive combinations (lynestrenol + mestranol; norethynodrel + mestranol and norethindrone + mestranol) was examined after an acute or chronic treatment over a range of doses to assess a dose-response relationship. The combinations were always given with fixed ratios of progestogen to oestrogen of 1:0·06; 1:0·015 and 1:0·05 respectively, to compare with ratios used in woman.

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