These data indicate that 4-phenylsemicarbazides derived from 1,1-dialkylated hydrazines possess anticonvulsant activity; however, it is of a lower order than that shown by 4-phenylsemicarbazides derived from 1,1,2-trialkylated hydrazines (1, 3). The cage compounds IIIq, IIIr, and IIIs were uniformly inactive and these results are consistent with the poor activity found for other cage compounds (4, 5).

EXPERIMENTAL[1]

Ethyl (1-Methylhydrazino)acetate—A solution of 16.7 g (0.1 mole) of ethyl bromoacetate in 17 ml of benzene was added dropwise with magnetic stirring over a 90-min period to a solution of 9.2 g (0.2 mole) of methylhydrazine in 50 ml of benzene. After the reaction mixture had stirred overnight at room temperature, the benzene phase was decanted and the salt residue was washed three times with 15 ml of benzene. The benzene was distilled through a 1-ft Vigreux column at 42 mm Hg. The residue was then distilled and afforded 8.82 g (67%) of a colorless oil, bp 88° (21 mm); IR (film): 1740 cm^{-1} (C=O).

Anal.—Calc. for $C_5H_{12}N_2O$: C, 45.44; H, 9.15; N, 21.20. Found: C, 45.29; H, 9.26; N, 21.31.

[1] Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. The IR spectra were taken on a Perkin-Elmer 700 spectrophotometer as either liquid films or potassium bromide pellets. NMR spectra were recorded on a Varian EM-360 or T-60 spectrometer, using tetramethylsilane as the internal reference. Mass spectra were obtained on a RMU-7 double-focusing spectrometer by Hitachi/Perkin-Elmer. Elemental analyses were performed by Baron Consulting Co., Orange, Conn. All compounds exhibited ^{1}H-NMR and mass spectra consistent with the structures shown.

4-Phenylsemicarbazides (III)—Compound IIIb was prepared by the dropwise addition of a solution of 2.66 g (0.0180 mole) of 2,6-dimethylphenyl isocyanate (I) in 6 ml of dry benzene to a solution of 1.20 g (0.020 mole) of 1,1-dimethylhydrazine in 10 ml of dry benzene at room temperature. After ~10 min heat was evolved. The mixture was heated for 2.5 h in an oil bath (85°). The solvent was evaporated under reduced pressure, and the residue was recrystallized from ethyl acetate and gave 3.40 g (91%) of white crystalline product, mp 156–158°.

REFERENCES

(1) M. J. Kornet, *J. Pharm. Sci.*, **67**, 1471 (1978).

(2) M. J. Kornet and R. Joyce Garrett, *J. Pharm. Sci.*, **68**, 377 (1979).

(3) M. J. Kornet and J. Chu, *J. Heterocycl. Chem.*, **18**, 293 (1981).

(4) M. J. Kornet and J. Chu, *J. Heterocycl. Chem.*, **19**, 697 (1982).

press.

(5) M. J. Kornet and J. Chu, *J. Pharm. Sci.*, **72**, 94 (1983).

(6) E. Schenker, Fr. 1,514,454; through *Chem. Abstr.*, **70**, 106529q (1969).

(7) R. B. Moffett, G. N. Evenson, and P. F. Von Voigtlander, *J. Heterocycl. Chem.*, **14**, 1231 (1977).

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Investigation of the β-Cyclodextrin–Hydrocortisone Inclusion Compound

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Abstract □ The formation of an inclusion compound by β-cyclodextrin with hydrocortisone has been studied by proton magnetic resonance (^{1}H-NMR) and phase solubility analysis. The magnitude of the chemical shifts of the interior and exterior β-cyclodextrin protons in the presence of hydrocortisone indicated that hydrocortisone is included within the β-cyclodextrin cavity and probably interacts with protons on the edge of the torus. The overall stoichiometry of the inclusion compound was not a single, simple relationship, but was unusual in that it was variable and apparently dependent on the relative amounts of hydrocortisone and β-cyclodextrin in the system.

Keyphrases □ Inclusion complexes—β-cyclodextrin–hydrocortisone, phase solubility analyses, ^{1}H-NMR □ β-Cyclodextrin–hydrocortisone—inclusion complexes, phase solubility analyses, ^{1}H-NMR □ Phase solubility analyses—β-cyclodextrin–hydrocortisone, ^{1}H-NMR

The formation of an inclusion compound by dinoprostone (prostaglandin E_2) with β-cyclodextrin has been reported earlier (1). From phase solubility analysis and ^{1}H-NMR spectroscopy it was concluded that a 1:1 complex formed, with the dinoprostone molecule partially included within the β-cyclodextrin cavity and the remainder of the molecule extended to the exterior of the torus.

In the present study attention has been directed to the formation of an inclusion complex between hydrocortisone and β-cyclodextrin. Such an inclusion compound by itself is not necessarily unique; however, these initial studies indicated that an unusual dependence apparently existed between the stoichiometry of the interaction and the concentration of β-cyclodextrin.

EXPERIMENTAL

The experimental procedure was similar to that employed previously (1). β-Cyclodextrin[1] was recrystallized twice from distilled water and dried under vacuum at 60°; hydrocortisone USP[2], was used as received; and water was double-distilled and deionized. Samples for ^{1}H-NMR spectroscopy were prepared by saturating a 2% w/v solution of β-cyclodextrin in D_2O[3] with hydrocortisone. Excess complex was allowed to precipitate and the supernatant solution of the inclusion compound was decanted. ^{1}H-NMR spectra at 100 MHz[4] were determined on the supernatant in standard 5-mm tubes.

Samples for phase solubility analysis were prepared by placing excess quantities of hydrocortisone (0.021, 0.040, or 0.060 g) with increasing amounts of β-cyclodextrin into 20-ml culture tubes containing 10 ml of water. The samples were sealed (with screw caps[5]) and rotated end-over-end at ~41 rpm for 24 hr in a thermostated water bath at 30 ± 0.1°. Aliquots of the supernatant were filtered through a prerinsed membrane filter (0.45 μm)[6] and spectrophotometrically assayed at 248 nm.

[1] Nutritional Biochemicals, Inc.
[2] Calbiochem.
[3] Bio-Rad Laboratories (99.85 mole % D_2O).
[4] Varian XL-100 NMR spectrometer.
[5] Teflon lined.
[6] Millipore Corp., Type HA.

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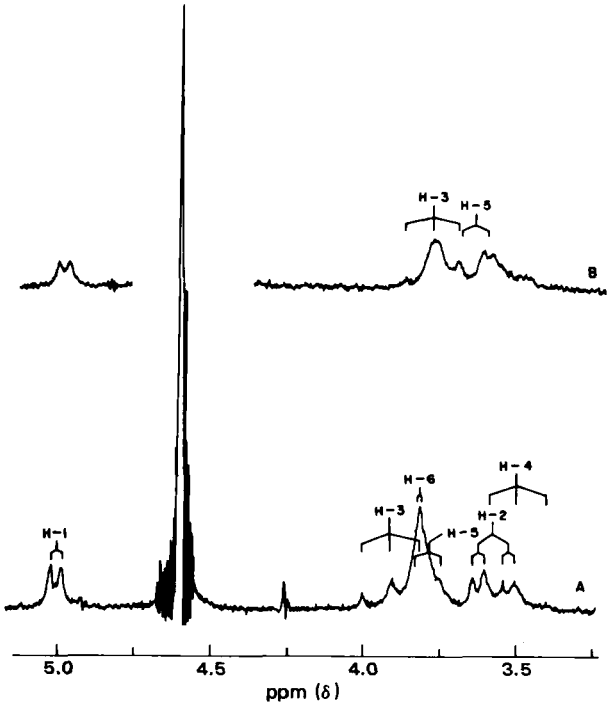

Figure 1—*^{1}H-NMR spectra of β-cyclodextrin (A) and the β-cyclodextrin–hydrocortisone inclusion compound (B) in D_2O.*

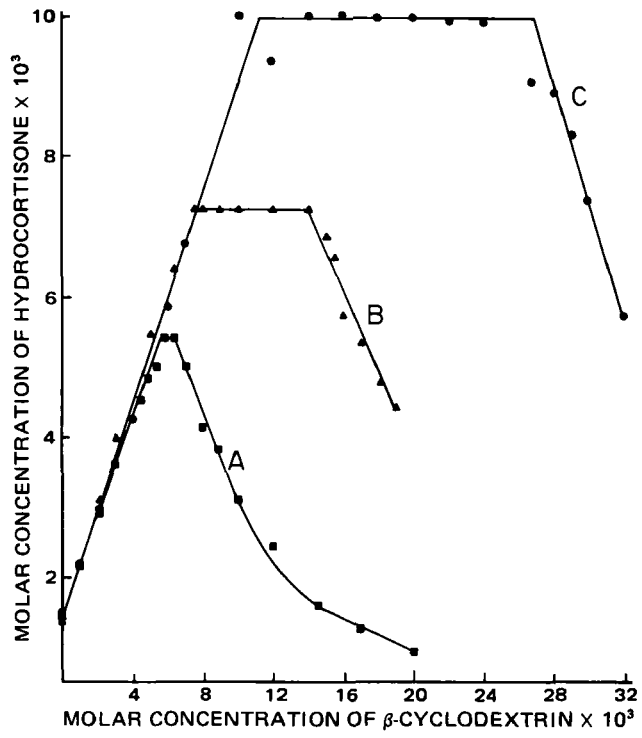

Figure 2—*Phase solubility diagram of β-cyclodextrin–hydrocortisone inclusion compounds at 30 ± 0.1°. Concentrations of hydrocortisone in excess of its solubility (per 10 ml of solution) and the stoichiometries of the soluble complexes (in moles of β-cyclodextrin/mole of hydrocortisone) were: A, 0.021 g and 1.4; B, 0.040 g and 1.7; and C, 0.060 g and 2.4, respectively.*

RESULTS AND DISCUSSION

The ^{1}H-NMR spectra of β-cyclodextrin and of the β-cyclodextrin–hydrocortisone inclusion compound are shown in Fig. 1. The β-cyclodextrin spectrum (Fig. 1A) is presented as a continuous spectrum; however, the spectrum of the inclusion compound (Fig. 1B) is broken for ease in comparison of the two spectra. Except for the HDO and HOH peaks, at ~4.6 ppm in both spectra, all of the peaks shown in Fig. 1 are those of β-cyclodextrin. In the case of the β-cyclodextrin–hydrocortisone inclusion compound, the hydrocortisone protons would be expected to appear further upfield, but due to their low concentration they were not detected.

From NMR and X-ray studies, the glucose units of β-cyclodextrin have been found to be in the C-1 chair configuration (2, 3), with primary and secondary hydroxyl groups around the opening of the torus. H-1, H-2, and H-4 are on the exterior and H-3, H-5, and possibly H-6 are located within the cavity. Other studies have shown that H-3 and H-5 undergo shielding by the guest component upon inclusion compound formation (3).

In the presence of hydrocortisone, the β-cyclodextrin spectrum is shifted upfield and reduced in magnitude due to the lower solubility of the inclusion compound compared with that of β-cyclodextrin alone. Similar behavior was found earlier for the dinoprostone–β-cyclodextrin systems (1). The anomeric hydrogens, H-1, appear as a doublet at the farthest downfield position and are gauche, whereas the rest of the β-cyclodextrin protons are axial. Due to their fixed position, the HDO and HOH peaks were used as internal standards in the measurement of the chemical shifts of the β-cyclodextrin protons in the presence of hydrocortisone, relative to their positions in the absence of hydrocortisone. Still farther upfield is a sideband (Fig. 1A), followed by a group of peaks corresponding to the remaining β-cyclodextrin protons. The sideband appeared only in the β-cyclodextrin spectrum.

The chemical shifts of the β-cyclodextrin protons in the presence of hydrocortisone are given in Table I. The H-3 and H-5 protons, which are located within the β-cyclodextrin cavity, show significant chemical shifts, and therefore underwent the greatest shielding effect due to the presence of the guest component. By contrast, H-1 and H-6 show much lower shifts, indicating a lower probability of interaction with hydrocortisone. The greater chemical shift of the H-5 protons compared with that of the H-3 protons suggest that on the ^{1}H-NMR time scale, more hydrocortisone entered the cavity from the H-5 side of the torus than from the H-3 side. Inclusion compounds of this type can be expected to occur by penetration from either side; however, complexes formed with hydrocortisone partially included on the H-5 side are apparently in greatest abundance. Although the stoichiometry of the β-cyclodextrin–hydrocortisone complex cannot be determined conclusively from ^{1}H-NMR observations, the strong chemical shifts of H-3 and H-5 suggest that the included portion of one hydrocortisone molecule fits snugly within the cavity, in close proximity to these protons. This observation is supported by a CPK space-filling model of the inclusion compound, which suggests that more than one molecule of hydrocortisone per molecule of β-cyclodextrin (each partially within the cavity) would result in a poor fit of either guest molecule, giving a very loosely bound complex.

Table I—Hydrocortisone-Induced Chemical Shifts of β-Cyclodextrin Protons

Proton	Δδ, ppm
H-1	0.03
H-2	0.01
H-3	0.11
H-4	0.01
H-5	0.13
H-6	0.02

Phase solubility analysis indicated, however, that the inclusion compound was not of simple 1:1 stoichiometry, but that the nature of the complex apparently was dependent on the total amount of hydrocortisone present relative to that of β-cyclodextrin (Fig. 2). While the general characteristics of portions of the phase solubility diagram in Fig. 2 follow that of the *p*-aminobenzoic acid–caffeine system reported by Higuchi and Lach (4), the molar concentration of hydrocortisone at the plateau region anomalously increased as the total amount of hydrocortisone increased. The approximate stoichiometries of the three β-cyclodextrin–hydrocortisone inclusion compounds represented by curves A, B, and C in Fig. 2 are 1.4, 1.7, and 2.4 molecules of β-cyclodextrin per molecule of hydrocortisone, respectively. Obviously these are difficult systems to interpret, and additional studies are in order to confirm the nature of the complex(es) and the mechanism of formation.

REFERENCES

(1) S. G. Frank and M. J. Cho, *J. Pharm. Sci.*, **67**, 1665 (1978).

(2) A. Hybl, R. E. Rundle, and D. E. Williams, *J. Am. Chem. Soc.*, **87**, 2779 (1965).

(3) A. L. Thakkar and P. V. Demarco, *J. Pharm. Sci.*, **60**, 652 (1971).

(4) T. Higuchi and J. L. Lach, *J. Am. Pharm. Assoc., Sci. Ed.*, **43**, 465 (1954).

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Metabolism of Cathinone to *d*-Norpseudoephedrine in Humans

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Received August 7, 1981, from the *Department of Pharmacy, University of Nairobi, Nairobi, Kenya.* Accepted for publication September 16, 1982.

Abstract □ Cathinone, a potent psychostimulant isolated from young shoots of *Catha edulis* was given to four human volunteers. Examination of urine collected from the volunteers at predetermined intervals showed the presence of unchanged cathinone, *d*-norpseudoephedrine, and two unidentified basic substances. The observed biotransformation of cathinone to the less potent psychostimulant, *d*-norpseudoephedrine involves reduction of a ketone group to alcohol, a common metabolic pathway in humans.

Keyphrases □ Cathinone—metabolism to *d*-norpseudoephedrine in humans □ *Catha edulis*—metabolism in humans

Catha edulis Forsk grows well in several countries of Eastern, Central, and Southern Africa. Because of the psychostimulant and mental dependence associated with habitual chewing of young shoots of this plant, it has been investigated extensively. Although chemical investigation of *C. edulis* goes back to the 19th century, the most significant result was obtained by Wolfes in 1930 (1) when he isolated and identified *d*-norpseudoephedrine, a CNS stimulant with amphetamine-like properties. There was some controversy as to whether the amount of *d*-norpseudoephedrine present in *Catha* material could account wholly for the observed psychostimulant effect, thus providing impetus for further chemical investigation (2). Several years later, a second phenylalkylamine closely related to *d*-norpseudoephedrine was isolated and named "cathinone" (3). This compound is a much more potent psychostimulant than *d*-norpseudoephedrine, and has attracted much attention as a potential drug of abuse (4).

The excretion of *d*-norpseudoephedrine in human urine has been investigated (5). In preliminary investigations involving known habitual chewers of *C. edulis*, *d*-norpseudoephedrine was shown to be the major compound present in urine when it was collected several hours after chewing *C. edulis*. Since cathinone is a major constituent of the young shoots of *C. edulis*, it seemed odd that it was absent or present only in trace amounts in several urine samples examined. A literature survey showed that the fate of cathinone in humans has not been investigated and accordingly this work was undertaken.

EXPERIMENTAL

Collection of Urine Samples—Four volunteers who had never chewed the material before participated in the experiment. Two volunteers were given 16 mg of cathinone extracted from young shoots of *C. edula* by preparatory TLC (3, 6), while the other two were given 16 mg of synthetic cathinone. All four volunteers had only milk for breakfast. Urine samples were collected at the following intervals: 0 (control), 0–4, 4–8, 8–12, 12–15, and 15–24 hr. Each urine sample was examined for the presence of cathinone and related substances as described below.

Examination of Urine for Cathinone, *d*-Norpseudoephedrine, and Related Basic Substances— Approximately 40 ml of urine was taken, 2–3 ml of strong lead acetate solution (7) was added, and after thorough mixing the precipitated proteins were removed by centrifugation. The supernatant was acidified to pH 5–6 with 0.1 *N* sulfuric acid and any precipitate removed by centrifugation. The acidified urine sample was extracted with chloroform, four times to remove acids and neutrals. The aqueous urine portion was then alkalinized (pH 9) using dilute ammonia solution and extracted with 4 × 100 ml of chloroform. The combined chloroform was distilled off at 40° using a rotary evaporator and the residue examined by TLC.

Examination of residue for *d*-norpseudoephedrine, cathinone, and related basic substances using TLC was carried out as follows. The residues were taken up in 2 ml of chloroform and ~10 μl was spotted on TLC plates coated with silica gel[1], and activated at 100° for 1 hr. Cathinone and *d*-norpseudoephedrine were spotted on the same TLC plates as the basic residues from urine. The plates were developed in either of the following solvent systems: (*A*) ethyl acetate–methanol–ammonia (17:2:1) or (*B*) cyclohexane–chloroform–diethylamine (5:4:1). Usually the plates were developed for 35–45 min. After development the plates were examined under UV light, then sprayed with 0.3% ninhydrin solution (8) after which they were heated at 105° for 10 min. The intensity of fluorescence under UV light and that of color after spraying with ninhydrin solution were judged on a 3-point scale as shown in the results. Where cathinone or *d*-norpseudoephedrine (or both) was not detected, the chloroform basic residue was concentrated almost to dryness and the experiment repeated.

The presence of *d*-norpseudoephedrine and cathinone in the basic residue extracted from urine was also investigated with a gas–liquid chromatograph[2] equipped with a flame ionization detector. The GLC column used was glass, 4-mm i.d. × 1.5 m long, packed with nonacid washed Chromosorb W (100–200 mesh)[3] coated with 1% polyethylene glycol 20 *M* (carbowax 20 *M*)[4]. The column was conditioned for 8–10 hr at 230°. The experimental operating conditions were as follows: hydrogen pressure, 1.4 kg/cm^2; condensed air pressure, 0.65 kg/cm^2; nitrogen flow rate 30 ml/min; column temperature programmed from 80–200° at 5°/min and finally left at 200° for 10 min; chart speed, 1 cm/2 min. The retention times and peaks were recorded[5].

For quantitative analysis, the areas under the curves were measured by the method of triangulation (9) and compared with areas obtained with standard solutions of cathinone and *d*-norpseudoephedrine. The standard solutions were prepared by dissolving 5 mg of either cathinone or *d*-norpseudoephedrine in 40 ml of control urine and subjecting this to the same extraction procedure as the experimental urine. It was then assumed that the percentage recovery of both compounds from experi-

[1] GF254, Merck & Co.
[2] Pye Unicam Series 104.
[3] Sigma Chemical Co.
[4] British Drug House.
[5] Pye Unicam linear recorder, type AR55.

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