in Q^F and T, the calculated percentages all tend to converge toward 100% as dosing increases. For example, in the sixth dosing cycle of Table IV + and −50% errors in k_{el} yield $\%C^{\tau}_{1,(n)}$ values of 68.7 and 173%, respectively, while ±50% errors in Q^F and T produce $\%C^{\tau}_{1,(n)}$ values of 92.7–107.3%. Similar results are found in Tables V and VI for $\%C^{max}_{1,(n)}$ and $\%AUC|^{\tau}_{0,(n)}$, respectively.

The effect of ±50% errors in k_{el} during multiple-dose cycles is further illustrated in Fig. 6 for six successively higher doses over 24 hr. The shapes of the plasma profiles are obviously distorted when compared with the error-free profile in Fig. 5. The pronounced effect of a −50% error in k_{el} is evidenced by values of $C^{max}_{1,(n)}$ and $C^{\tau}_{1,(n)}$ that begin to exceed the hypothetical toxic level of 5 μg/ml after only five dose cycles. Values of $C^{max}_{1,(n)}$ and $C^{\tau}_{1,(n)}$ for a +50% error in k_{el} all safely fall within the therapeutic region over the total 24 hr as expected. The increasingly larger differences between the areas under the curve for ±50% errors in k_{el} as the dose cycle increases are immediately apparent.

It may be concluded that individual patient differences in elimination, as reflected in k_{el}, become increasingly more important as a source of error in $C^{max}_{1,(n)}$, $C^{\tau}_{1,(n)}$, and $AUC|^{\tau}_{0,(n)}$ with successively higher doses while experimental error in Q^F and T become less and less important.

Finally, it is cautioned that the attainment clinically of profiles as ideal as that in Fig. 5 depends on an accurate estimation of the pharmacokinetic parameters for the individual patient, a constancy in the values of the pharmacokinetic parameters during therapy, and careful administration of the drug with respect to both flow rate and T.

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Synthesis and Biological Activity of Cocaine Analogs I: *N*-Alkylated Norcocaine Derivatives

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Abstract □ *N*-Allylnorcocaine, *N*-dimethylallylnorcocaine, and *N*-cyclopropylmethylnorcocaine were prepared and examined for cocaine-like activity. The compounds were prepared by alkylation of norcocaine, which was obtained by demethylation of cocaine with 2,2,2-trichloroethyl chloroformate followed by zinc–acetic acid reduction. The compounds were evaluated by comparison with cocaine in causing disruption of milk intake in rats, behavioral modification in squirrel monkeys, and inhibition of ^{3}H-serotonin uptake by rat synaptosomes. The compounds showed cocaine-like activity less potent than cocaine in the latter two tests and were inactive in the milk intake test.

Keyphrases □ Cocaine analogs, various—synthesized, evaluated for effect on milk intake in rats, behavioral modifications in monkeys, and effect on ^{3}H-serotonin uptake by rat synaptosomes □ Structure–activity relationships—various cocaine analogs evaluated for effect on milk intake in rats, behavioral modifications in monkeys, and effect on ^{3}H-serotonin uptake by rat synaptosomes

Structural modifications of cocaine have been undertaken to derive compounds with the ability to interact at the cocaine receptor but with a lower intrinsic activity. Such an approach ultimately may lead to compounds with the ability to antagonize the euphoric effects of cocaine (I) as an adjunct to the treatment of cocaine abuse. Another desirable effect would be to attenuate the potent central nervous system (CNS) stimulant properties (1) of cocaine to obtain mood elevation without the prolonged latency period of tricyclic antidepressants and monoamine oxidase inhibitors. Although not defensible on any theoretical basis, previous reports on narcotic analgesics (2–6), CNS stimulants (7), and hallucinogens (8) strongly suggested the synthesis of *N*-allylnorcocaine[1] (IV), *N*-dimethylallylnorcocaine (V), and *N*-cyclopropylmethylnorcocaine (VI). This paper reports the syntheses and evaluations of IV–VI.

EXPERIMENTAL[2]

Norcocaine (III) was prepared by treatment of carbamate II with zinc and 95% acetic acid. The carbamate (II) was obtained by treating cocaine with 2,2,2-trichloroethyl chloroformate (10) and potassium carbonate in refluxing benzene. The procedure varied slightly from that described by Borne *et al.* (11) and provided similar yields. Alkylated norcocaine derivatives IV–VII were prepared by treating III with the appropriate alkyl bromide and triethylamine in refluxing benzene. Cyclopropylmethyl bromide, the only alkyl bromide not available commercially, was prepared by treating cyclopropylcarbinol with triphenylphosphine and bromine in dimethylformamide.

N-Cyclopropylmethylnorcocaine (VI) was purified by column chro-

[1] While the biological studies on these compounds were being carried out, the synthesis of *N*-allylnorcocaine and its effects on temperature, respiration, and heart rate in monkeys were reported (9).

[2] Melting points were determined on a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Elemental analyses were performed by Baron Consultants, Orange, Conn. NMR spectra were obtained on a Hitachi Perkin-Elmer R-24 high-resolution NMR spectrometer using deuterated chloroform as the solvent. TLC was run on silica gel GF-254 (type 60) (EM Reagents). Column chromatography was run on silica gel 60 with a particle size of 0.063–0.200 mm (70–230 mesh ASTM) (EM Reagents). Benzene–ethanol–ammonium hydroxide (10:1:1), top phase, served as the solvent system for all chromatography.

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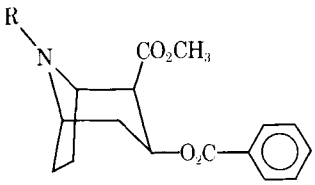

I: R = CH_3
II: R = $CO_2CH_2C(Cl)_3$
III: R = H
IV: R = $CH_2CH{=}CH_2$
V: R = $CH_2CH{=}C(CH_3)_2$
VI: R = CH_2—⟨△⟩
VII: R = $CH_2CH_2CH{=}CH_2$

matography because of formation of a small amount (5–10%) of side product. The identity of the side product, *N*-(3-butenyl)norcocaine (VII), was determined by isolation of a small amount from the column and comparison (TLC and NMR) with a separately prepared sample. The homoallylic by-product probably forms by S_N2' attack of III on cyclopropylmethyl bromide. No homoallyl bromide was detectable (NMR) in the cyclopropylmethyl bromide.

The norcocaine derivatives were tested in three biological systems: milk intake in rats, ^{3}H-serotonin uptake in rat brain synaptosomal preparations, and behavioral effects on squirrel monkeys.

Norcocaine (III)—To a stirred refluxing mixture of cocaine (8 g, 0.026 mole), potassium carbonate (0.8 g, 0.0058 mole), and 140 ml of dry benzene, 2,2,2-trichloroethyl chloroformate (16 g, 0.0757 mole) was added over 30 min. The reaction mixture was refluxed for 40 hr. It was then cooled and poured into 150 ml of ice water. The benzene layer was separated, and the aqueous layer was extracted thoroughly with chloroform. The combined organic layers were washed with saturated sodium chloride solution, dried over anhydrous sodium sulfate, and evaporated to yield II as an oil.

Without further purification, II was dissolved in 130 ml of 95% acetic acid. Zinc dust (20 g, 0.306 mole) was added slowly. The mixture was stirred for 23 hr and then filtered. The zinc salts were rinsed with a small amount of 95% acetic acid. The filtrate was diluted with 450 ml of water and extracted thoroughly with chloroform. The combined chloroform extracts were then washed with 5% sodium hydroxide solution until the aqueous phase remained basic. The organic phase was washed with water, dried over anhydrous sodium sulfate, and evaporated to give norcocaine as an oil.

A small amount of the oil was triturated in cold petroleum ether. The petroleum ether was decanted, and the process was repeated until the norcocaine solidified. Two recrystallizations from petroleum ether gave norcocaine (III), mp 82.5–83.5° [lit. (12) mp 82°]. The remaining oil, without further purification, was converted to its hydrochloride salt, mp 115–117° (from acetone) [lit. (11) mp 111–113°]. Norcocaine hydrobromide, mp 164–165° (from acetone–ethanol), also was prepared. In several parallel runs, the overall yield (based on the yield of crude hydrochloride salt) ranged from 54 to 65%.

Cyclopropylmethyl Bromide—Cyclopropylmethyl bromide was prepared from cyclopropylcarbinol in 30% yield by the method used by Wiley *et al.* (13) to prepare *n*-butyl bromide from *n*-butyl alcohol. However, instead of an aqueous workup, the product was distilled, bp 95–115°/760 mm [lit. (14) bp 102–110°/760 mm]. Its NMR spectrum indicated that it still contained about 30% reaction solvent [dimethylformamide, δ 2.75 (s, CH_3) and 2.9 (s, CH_3) ppm]. The crude cyclopropylmethyl bromide was used in the alkylation reaction with norcocaine without further purification.

General Procedure for Alkylation of Norcocaine—N-*Dimethylallylnorcocaine Hydrochloride* (*V Hydrochloride*)—A solution of norcocaine (1.15 g, 0.004 mole), 1-bromo-3-methyl-2-butene (0.59 g, 0.004 mole), triethylamine (0.6 g, 0.006 mole), and 10 ml of anhydrous benzene was refluxed. The progress of the reaction was monitored by TLC at 1- or 2-hr intervals. At each interval, a few drops of the alkyl bromide was added. After 7 hr, TLC indicated that all of the norcocaine had reacted. The reaction mixture was then diluted with 10 ml of ether, and the mixture was filtered and evaporated under reduced pressure to afford an oil.

The oil was dissolved in ether and extracted into 1.5 *N* HCl. The aqueous solution was made basic with 6 *N* NaOH, and the product was extracted back into ether. The ether layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure to give V as an oil. The hydrochloride salt was prepared by dissolving the oil in anhydrous ether and adding an ethereal solution of hydrochloric acid. In several parallel runs, the yields ranged from 67 to 72% of hydrochloride, mp 161.5–162° (from acetone); NMR: δ 1.85 (6H, s, CH_3), 3.76 (3H, s, OCH_3), and 7.4–8.1 (5H, m, aromatic) ppm.

Anal.—Calc. for $C_{21}H_{18}ClNO_4$: C, 64.03; H, 7.17; N, 3.56. Found: C, 63.75; H, 7.06; N, 3.69.

N-*Allylnorcocaine Hydrochloride* (*IV Hydrochloride*)—This compound was obtained in 60–67% yield, mp 154–155°; NMR: δ 5.35–5.9 (4H, m, vinyl and CHOCO-phenyl), 3.75 (3H, s, OCH_3), and 7.4–8.1 (5H, m, aromatic) ppm.

Anal.—Calc. for $C_{19}H_{24}ClNO_4$: C, 62.38; H, 6.61; N, 3.83. Found: C, 62.18; H, 6.66; N, 3.80.

N-*Cyclopropylmethylnorcocaine Hydrochloride* (*VI Hydrochloride*)—The hygroscopic salt, mp 98–103°, was obtained in 54–80% yield after purification of the free base by column chromatography to remove VII, which was identified by comparison (NMR and TLC) with an authentic sample; NMR: δ 0.46–0.82 (5H, m, cyclopropyl), 3.78 (3H, s, OCH_3), and 7.4–8.1 (5H, m, aromatic) ppm.

Anal.—Calc. for $C_{20}H_{26}ClNO_4$: C, 63.24; H, 6.90; N, 3.69. Found: C, 62.95; H, 6.80; N, 3.81.

N-(*3-Butenyl*)*norcocaine Hydrochloride* (*VII Hydrochloride*)—This compound was obtained in 50% yield, mp 125–127°; NMR: δ 4.9–6.0 (4H, m, vinyl and CHOCO-phenyl), 3.75 (3H, s, OCH_3), and 7.4–8.2 (5H, m, aromatic) ppm.

Anal.—Calc. for $C_{20}H_{26}ClNO_4$: C, 63.24; H, 6.90; N, 3.69. Found: C, 62.86; H, 6.76; N, 4.00.

Biological Methods—In the milk intake studies, eight experimentally naive female Sprague–Dawley-derived rats weighed between 210 and 290 g at the beginning of the experiments. They were housed individually in stainless steel cages; water was available at all times except during experimental sessions. Experimental sessions consisted of 15 min/day access to a sweetened condensed milk solution (two parts of tap water to one part of commercial sweetened condensed milk[3]), which was placed on the front of the cage in a graduated drinking tube with a drinking spout. The volume of milk intake was measured at the end of the session. Each rat received 4–6 g of food after the session.

The effects of the experimental compounds on milk intake were determined in the following manner. After milk intake was stable for the group of rats (±10% for 3 days), each rat was injected 15 min before the session with saline or a dose of the test compound in a volume of 1 ml/kg ip. After dose–effect relations of all compounds had been determined, the effects of several doses of each compound plus cocaine were determined to test for possible antagonism of the effects of cocaine on intake. Interaction studies were carried out by injecting the test drug intraperitoneally 5 min before the intraperitoneal injection of cocaine, which preceded access to milk by 15 min.

For the inhibition of serotonin uptake study, subcellular fractions of rat brain (striatum hypothalamus, hippocampus, and midbrain) were prepared by the method of Gray and Whittaker (15). A crude nucleus fraction was obtained by centrifugation of 10% brain homogenate in 0.32 *M* sucrose at 1000×*g* for 10 min. The supernate was sedimented further at 12,000×*g* for 20 min to obtain the P2 pellet (crude mitochondria fraction). Synaptosomes and mitochondria were fractioned from resuspended P2 by a discontinuous sucrose gradient procedure (15). The P2B layer (containing the nerve ending particles) was aspirated, and the sucrose concentration was adjusted to 0.32 *M*. It then was resedimented at 12,000×*g* for 20 min to obtain the synaptosomal fraction.

The uptake of serotonin by isolated synaptosomes was measured by a modification of the procedure of Neckers and Sze (16). A 600-μl aliquot of the incubation mixture contained 0.5 nmole of unlabeled 5-hydroxytryptamine, 30 nmoles of test drug, 1 μCi of labeled biogenic amine, and synaptosomal preparation equivalent to 1.2–1.7 mg of protein resuspended in a modified Krebs–Ringer phosphate buffer, pH 7.4, containing 12 m*M* glucose and 0.08 m*M* pargyline hydrochloride. The mixture was allowed to incubate for 5 min at 30°. After incubation, the synaptosomal suspension was immediately cooled in ice for 2 min after the addition of ice-cold Ringer solution (3 ml) and centrifuged at 12,000×*g* for 15 min. The supernate was poured out, and the tubes were cleaned cautiously with cotton swabs without touching the pellet. The pellet was vortexed in 250 μl of 50% ethanol, and 100 μl was pipetted into the scintillation vial containing 10 ml of Bray's solution. Radioactivity in the samples was then measured[4].

[3] Borden.
[4] Packard Tri-Carb liquid scintillation spectrometer.

Table I—Inhibition of Milk Intake by Cocaine and Analogs in Rats

Compound (Hydrochloride Salt)	Dose, mg/kg	n	Mean Intake, ml	*SEM*, ml
Saline		8	29.5	0.4
I	4	4	25.5	2.3
	8	4	19.0	2.1
	16	4	22.0	1.2
	32	4	15.7	2.7
IV	4	7	20.1	0.87
	8	8	30.6	2.3
	16	8	27.2	2.2
	32	8	26.0	1.8
	64	4	20.5	2.2
V	4	4	31.6	3.3
	8	4	33.2	2.8
	16	4	26.1	3.8
	32	4	30.7	1.9
	64	2	26.0	0
VI	16	2	25	20–30
	32	2	24	22–26

Table II—Interaction of Cocaine and Analogs in Inhibition of Milk Intake in Rats

Compound (Hydrochloride Salt)	Dose, mg/kg	n	Mean Intake, ml	*SEM*, ml
Saline		8	29.5	0.4
I	16	4	22.0	1.2
IV + I	32 + 16	4	13.2	2.2
V + I	8 + 16	4	10.5	6.1
V + I	16 + 16	4	20.7	2.0
V + I	64 + 16	4	22.5[a]	3.0
V + I	64 + 32	4	0[b]	0
VI + I	32 + 16	1	6.0	—

[a] Convulsions in one rat; prostration in one rat. [b] Stereotypy, frothing, and intermittent convulsions in one rat.

To study the behavioral effects of the compounds on squirrel monkeys, IV and VI (0.3, 1.0, 10.0, and 30.0 mg/kg im) and V (0.3, 1.0, 3.0, and 10.0 mg/kg im) were administered to each of two mature male squirrel monkeys. Each monkey received a single administration of each dose, and successive administrations were separated by at least 48 hr. Two observers recorded the effects of each dose at various times after administration (1–60 min). Four other mature male squirrel monkeys responded under a multiple 5-min fixed-interval (FI), 30-response fixed-ratio (FR) schedule of termination of a stimulus associated with electric shock. A description of this test is given in detail elsewhere (17). Cocaine (0.1, 0.3, 1.0, and 3.0 mg/kg im) and IV–VI (0.3, 1.0, 3.0, and 10.0 mg/kg im) were administered 5 min before the beginning of a 75-min experimental session. Each monkey received duplicate administrations of each dose, and at least 48 hr separated successive administrations.

RESULTS AND DISCUSSION

Compounds IV–VI were compared to cocaine (I) for their ability to disrupt milk intake in rats (18, 19). They were also coadministered with cocaine to test for possible inhibition of cocaine-induced disruption of milk intake. Dose–effect results for each compound are presented in Table I. There was a dose-related decrease in milk intake following injections of cocaine. Compound IV showed weak activity at the lowest and highest doses tested but not in a dose-related manner. Compounds V and VI did not disrupt milk intake at a significant level at any dose tested.

The data for the interaction studies are shown in Table II. None of the compounds showed evidence of antagonism of the effects of cocaine. Differences between the combination of drugs and cocaine alone were in the direction of a greater disruption of intake, although these effects were not statistically significant because of high variability. With high doses (64 mg of V/kg with 32 mg of I/kg), milk intake was precluded completely due to convulsions and frothing.

Compounds IV–VI also were compared with cocaine in their ability to inhibit ^{3}H-serotonin uptake by synaptosomes isolated from the rat midbrain. The results (Table III) indicate that these compounds inhibited ^{3}H-serotonin uptake, as cocaine does. However, approximately 10 times the concentration of IV–VI was required to bring about the range of inhibition exhibited by cocaine.

Table III—Cocaine Derivatives as Inhibitors of ^{3}H-Serotonin Uptake in Rat Synaptosomal Preparations

Compound (5×10^{-5} *M*)[a] (Hydrochloride Salt)	Inhibition[b,c], %
Cocaine (5.3×10^{-6} *M*)	26
IV	25
V	15
VI	32.4

[a] Concentration unless otherwise stated. [b] The value for cocaine was an average value obtained over six experiments ($\bar{X}$ = 26.033, *SD* = 1.426). [c] The values for IV–VI were averages of two determinations.

In behavioral tests with squirrel monkeys, at the highest doses tested, V and VI produced convulsions almost immediately after injection (3 min or less) and continued to do so for up to 10 (V) or 30 (VI) min. The highest dose of IV produced marked incoordination and rapid and shallow respirations but not convulsions. Doses of 10.0 (IV and VI) or 3.0 (V) mg/kg or less produced no gross behavioral effects in either monkey.

Some doses of cocaine (0.3 and 1.0 mg/kg) or of IV–VI (1.0 and 3.0 mg/kg) increased responding under the FI schedule to 130–180% of control (control mean, 0.68 response/sec); the largest dose of cocaine (3.0 mg/kg) or of VI (10.0 mg/kg) decreased FI responding. Cocaine and IV–VI only decreased responding under the FR schedule (control mean, 2.14 responses/sec). The 3.0-mg/kg dose of cocaine and the 10.0-mg/kg dose of IV and VI decreased FR responding to about the same extent, 30–60% of control. The behavioral effects of IV–VI were qualitatively similar to those of cocaine, but each compound was less potent than cocaine.

In conclusion, these *N*-alkylated norcocaine derivatives exhibited cocaine-like activity in two of the three biological test systems. This result suggests that these or similar compounds can interact at the cocaine receptor. The fact that these compounds were less potent than cocaine is encouraging considering the possible implications mentioned earlier.

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