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β-Cocaine

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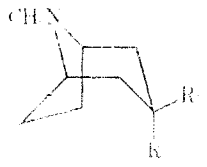
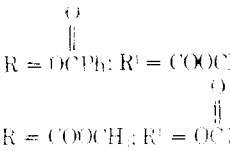
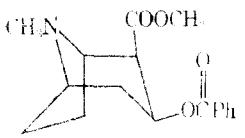
The present paper is the final chapter of a story of some 78 years duration in the chemical literature. In 1896 Willstätter[1] prepared a cocaine analog from 1αH,5αH-tropan-3-one called "α-cocaine." He published the correct structure for the tropane ring system 2 years later[2] and showed both substituents of α-cocaine to be on carbon-3 (structure **1a** or **1b**). Only one isomer was found. The compound produced no numbness of the tongue, an outstanding property of its relative, cocaine (**2**). Consideration of the fact that cocaine contains an equatorial 3-benzoyloxy group would lead one to assume that the nonanesthetic α-cocaine probably is represented by structure **1a** with its benzoyloxy group bent away from the amine side of the molecule rather than by **1b** wherein this group is equatorial.[3]



1a, R = OCPh; R' = COOCH$_3$



b, R = COOCH$_3$; R' = OCPh



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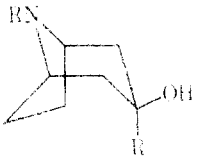
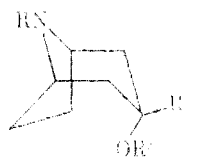
Foster and Ing[4] noted that the lack of local anesthetic activity observed with α-cocaine was based only on a topical test (production of tongue numbness) and surmised that it might show activity by subdermal infusion. They repeated Willstätter's synthesis, *i.e.*, tropanone → cyanohydrin → hydroxy acid → hydroxy ester → α-cocaine, again found the single isomer, and, together with Varagić,[5] demonstrated that it was one-third to one-eighth as active as cocaine in the guinea pig wheal test.[6] It was active but did not have the cocaine activity profile. The biological results certainly gave no satisfactory basis for structural assignment.

Heusner[3] settled the matter chemically, showing that α-cocaine is actually **1b**. He used 1αH,5αH-nortropan-3-one as a starting material, isolated a single crystalline cyanohydrin isomer and converted it ultimately to α-cocaine. Therefore, the matters of structure and activity were settled but no isomeric cyanohydrin had been found which could lead to "β-cocaine" (**1a**).

In this laboratory the cyanohydrin of 8-benzyl-1αH,5αH-nortropan-3-one was prepared. When the viscous, oily product could not be induced to crystallize, the crude material was hydrolyzed (concentrated HCl) and esterified (CH_3OH·HCl). The resulting mixture yielded 40% of recovered 8-benzyl-1αH,5αH-nortropan-3-one, 16% of methyl 8-benzyl-3β-hydroxy-1αH,5αH-nortropane-3α-carboxylate (*N*-benzylnor-α-ecgonine methyl ester, **3b**), and 20% of methyl 8-benzyl-3α-hydroxy-1αH,5αH-nortropane-3β-carboxylate (*N*-benzylnor-β-ecgonine methyl ester, **4b**).



3a, R = PhCH$_2$; R' = CN
b, R = PhCH$_2$; R' = COOCH$_3$
c, R = H; R' = COOCH$_3$



4a, R = PhCH$_2$; R' = CN; R'' = H
b, R = PhCH$_2$; R' = COOCH$_3$; R'' = H
c, R = PhCH$_2$; R' = COOCH$_3$; R'' = COPh
d, R = H; R' = COOCH$_3$; R'' = COPh
e, R = CH$_3$; R' = COOCH$_3$; R'' = COPh

The structures of isomers **3b** and **4b** were provisionally assigned on the basis of their nmr spectra. The nmr spectrum of **4b** showed a rather sharp (nonbonded) OH peak with δ 3.13 ppm and a benzylic singlet deshielded by COOMe at 3.62 ppm. The spectrum of **3b** showed a very diffuse bonded OH mound and a nondeshielded benzylic singlet at 3.45 ppm. The structures of these isomers were then firmly established by debenzylation of **3b** to the known nor compound **3c**[3] which was compared directly with an authentic sample (from 1αH,5αH-nortropan-3-one[7] kindly furnished by Dr. M. R. Bell of this Institute.

In seeking an explanation for the unexpected appearance of this α-hydroxy-β-carboxylic ester, it was recalled that the cyanohydrins from both 1αH,5αH-tropan-3-one and 1αH,5αH-nortropan-3-one are crystalline products which separate quickly and in high yield. If HCN addition under the extant reaction conditions is sufficiently reversible and if precipitation of crystalline β-hydroxy-α-nitrile simply shifts the equilibrium in its favor, simple filtration of the gratifyingly large yield of cyanohydrin would have precluded obtaining the other isomer. All workers to date used *crystalline* cyanohydrin. The happenstance of noncrystallinity of our cyanohydrin products seems to have allowed isolation of the isomer of opposite configuration.

With this unexpected precursor in hand, it was then possible to prepare β-cocaine. Attempted benzoylation of hydroxy ester **4b** with benzoic anhydride in refluxing THF produced no reaction. At 150–160° (no solvent) some α,β-unsaturated ester began to form with no sign (tlc) of benzoate ester. Treatment of **4b** with BuLi followed by benzoyl chloride produced benzoate ester **4c** in low yield but use of KH[7] instead of BuLi gave a 76% yield of this ester.

Debenzylation of **4c** (Pd/C) formed **4d**. N-Methylation of **4d** (HCOOH/HCHO) then furnished methyl 3α-hydroxy-1αH,5αH-tropane-3β-carboxylate 3-benzoate (**4e**), the long-sought β-cocaine.

After so much discussion over the years of differences in local anesthetic activity to be expected from the two structures isomeric about carbon-3, it is somewhat ironical that β-cocaine (**4e**) produces no numbness of the tongue and is about one-third as active as cocaine in the intradermal guinea pig wheal test,[6] the same activity shown by α-cocaine.[5]

Experimental Section

All melting points were determined in capillary tubes and are uncorrected. Nmr spectra were recorded on a Varian HA-100 spectrometer using TMS as an internal standard. Ir oil film spectra were recorded with a Perkin-Elmer 257 spectrometer and the ir spectra in KBr were recorded on a Perkin-Elmer Model 21 spectrometer. Analytical results for indicated elements are within ±0.4% of the theoretical values.

Methyl 8-Benzyl-3-hydroxy-1αH,5αH-nortropane-3α-carboxylate (3b) and Methyl 8-Benzyl-3-hydroxy-1αH,5αH-nortropane-3β-carboxylate (4b). A mixture of 215.3 g (1.00 mol) of 8-benzyl-1αH,5αH-nortropan-3-one and 200 ml of ice was treated with 85 ml of concentrated HCl, and ice was then added to give a volume of 550 ml. To the stirred mixture at 0–5° was added a cold solution of 66.7 g (1.02 mol) of KCN in 135 ml of H_2O in 5 min. It was stirred for 1 hr at 5° and let stand overnight at this temperature.

The aqueous layer was decanted and the residual viscous oil was rinsed with 15 ml of brine. The oil was then dissolved by cautious addition of 2 l. of concentrated HCl with cooling (20-ml increments initially). It probably would be better to add oil to the cold acid. The resulting solution was stored overnight at about 8°, boiled down to 60% of its volume over a 3-hr period (hot hydrolysis is necessary), and then concentrated to a paste by warming *in vacuo*. MeOH (1 l.) was added, a precipitate of KCl was removed, and the filtrate was concentrated at up to 90° *in vacuo*. The dark, tarry residue was dissolved in 2 l. of MeOH and the solution was saturated with gaseous HCl without external cooling. The process finally produced boiling. This solution was set aside for 16 hr and then concentrated by warming *in vacuo*. A pasty residue remained which was diluted with 150 ml of MeOH and filtered. The filter cake was washed with three 30-ml portions of cold MeOH and air-dried to give 81.0 g of largely 3β-ester **4b**·HCl together with some 8-benzyl-1αH,5αH-nortropan-3-one hydrochloride. Boiling this solid with 500 ml of absolute EtOH and hot filtration gave 47.5 g of practically pure **4b**, mp 229–230° dec. Cooling the filtrate to 25° gave a further 16.2 g of **4b**: mp 232–233° dec; total yield 20%. The mother liquor, containing 8-benzyl-1αH,5αH-nortropan-3-one and a trace of **3b**, was not worked up. The analytical sample of **4b**·HCl (plates from absolute EtOH) melted at 235–236° dec. *Anal.* ($C_{16}H_{21}NO_3$·HCl) C, H, Cl. The free base, liberated by concentrated NH_4OH, boiled at 134–135° (0.3 mm); $n^{25}D$ 1.5420; it solidified, mp 54.5–56° (prisms from pentane); ir λ_{max} (oil) 2.85 (sharp) and 5.79 μ; nmr ($CDCl_3$) δ 3.13 (sharp, OH), 3.62 ppm (s, 2, $PhCH_2$), aromatic region complex; R_f silica gel 0.45 (20:2:78 pentane-*i*-$PrNH_2$-Et_2O).

The filtrate from the collection of the 81 g above was concentrated and the residue was treated with excess concentrated NH_4OH. Extraction of the basic material with ether and distillation gave 110 g of oil boiling at 135–164° (0.7 mm) and 39.2 g of oil boiling at 164–166° (0.7 mm). The second fraction was diluted with 50 ml of pentane whereupon it crystallized and afforded 32.2 g of **3b**, mp 88–90°. Recrystallization from Et_2O gave the analytical sample: mp 90.5–92°; ir λ_{max} (oil) 2.93 (broad) and 5.76 μ; nmr ($CDCl_3$) δ 3.48 (s, 2, $PhCH_2$), aromatic region simple; R_f silica gel 0.25 (20:2:78 pentane-*i*-$PrNH_2$-Et_2O). *Anal.* ($C_{16}H_{21}NO_3$) C, H, N.

Table I. Local Anesthetic Activity in Guinea Pigs

	Concn, %[a]	No. positive/no. tested	Mean anesthetic score	TAC_5 %, g/ml	Act. ratio[b]
Cocaine	0.125	4/4	27		
	0.06	4/4	14	0.031	
	0.03	3/4	5		
β-Cocaine	0.5	6/6	27		
	0.25	6/6	21	0.092	0.33
	0.125	6/6	8.5		

[a] The concentrations shown are those of the hydrochloride salts of these compounds. [b] Activity of cocaine = 1.

Careful fractionation of the 110 g of the first fraction above gave 85.8 g (40%) of 8-benzyl-1αH,5αH-nortropan-3-one, bp 125–138° (0.6 mm) (with termination of the distillation when the temperature began to rise sharply). Dilution of the pot residue with 50 ml of pentane and seeding gave a sticky solid. Multiple trituration of this solid with 1:1 Et_2O–pentane afforded 12.4 g more of clean (tlc) **3b**, total yield 44.6 g (14%).

Methyl 3-Hydroxy-1αH,5αH-nortropane-3α-carboxylate (3c).[3] A solution of 0.37 g (1.35 mmol) of **3b** in 100 ml of EtOH and 0.75 ml (1.5 mmol) of 2 *N* HCl was treated with H_2 under 3.5 kg/cm² for 2 hr in the presence of 0.20 g of 10% Pd/C catalyst. Removal of catalyst and solvent, treatment of the residue with concentrated NH_4OH, and extraction with Et_2O gave 0.28 g of crystalline **3c**. Two recrystallizations from EtOAc gave diamond-shaped plates which underwent a polymorphic transformation to long prisms upon standing cold, mp 145–148° and undepressed upon admixture with an authentic sample prepared from 1αH,5αH-nortropan-3-one by Dr. M. R. Bell. The identity was further supported by ir and nmr spectral comparisons.

Methyl 8-Benzyl-3-hydroxy-1αH,5αH-nortropane-3β-carboxylate 3-Benzoate *p*-Toluenesulfonate (4c). A solution of 2.75 g (0.010 mol) of hydroxy ester **4b** in 10 ml of THF under N_2 was treated dropwise with stirring in 2 min with 1.20 g of 50% KH in oil (0.60 g or 0.015 mol of KH). The temperature rose to 40° and frothing subsided in 5 min. After 30 min, 1.70 g (0.012 mol) of benzoyl chloride was added in one portion. After 1 hr the gelatinous mixture was diluted with Et_2O and treated cautiously with 5 ml of H_2O. The Et_2O layer was washed with brine and concentrated to give 3.43 g of oily product. Treatment of this product with 1.9 g (0.010 mol) of *p*-toluenesulfonic acid monohydrate furnished 4.20 g (76%) of crystalline **4c**·tosylate salt. Recrystallization from 100 ml of absolute EtOH concentrated to a 30-ml volume gave 3.2 g of colorless needles: mp 238–240°; λ_{max} (KBr) 5.76 and 5.82 μ; nmr (DMSO-d_6) compatible with structure. *Anal.* ($C_{23}H_{25}NO_4$·$C_7H_8O_3S$) C, H, S.

Methyl 3-Hydroxy-1αH,5αH-nortropane-3β-carboxylate 3-Benzoate *p*-Toluenesulfonate (4d). To a partial solution of 8.3 g (0.015 mol) of **4c** in 300 ml of 95% undenatured EtOH was added 1.0 g of 10% Pd-on-charcoal catalyst. This mixture was treated with H_2 under 3.5 kg/cm² of pressure for 7 hr at room temperature. H_2 (1 equiv) was absorbed. Removal of the catalyst and solvent and addition of 5 ml of absolute EtOH and 50 ml of Et_2O caused precipitation of 6.6 g (96%) of blade clusters of **4d**, mp 178–181°. The analytical sample had mp 180.5–182° (EtOH). *Anal.* ($C_{16}H_{19}NO_4$·$C_7H_8O_3S$) C, H, S.

β-Cocaine (Methyl 3-Hydroxy-1αH,5αH-tropane-3β-carboxylate 3-Benzoate) (4e). A solution of 3.95 g (0.014 mol) of **4d** in 80 ml of 98% HCOOH and 24 ml of 35% aqueous HCHO was heated at 100° for 2 hr and then concentrated to a residue by warming *in vacuo*. The residue was treated with dilute NaOH and the basic product extracted with Et_2O. Concentration of the extracts gave 2.34 g (54%) of colorless oil: λ_{max} (film) 5.74 and 5.84 μ; nmr ($CDCl_3$) δ 2.35 (s, 3, NCH_3) and 3.7 ppm (s, 3, OCH_3). The HCl salt of this base formed heavy needles (CH_3CN), mp 221–222° dec with heating rate of 3°/min. The melting point varies with heating rate. α-Cocaine hydrochloride melts at 180°.[4] *Anal.* ($C_{17}H_{21}NO_4$·HCl) C, H, Cl.

Biology. For determination of the action of β-cocaine hydrochloride in producing topical anesthesia of the tongue, 1 mg of the powder was rubbed onto the tip of the human tongue and kept there undisturbed for 1 min. Then the mouth was rinsed and the development of a numbness was evaluated over a 10-min period.

Cocaine hydrochloride produced a marked numbness. β-Cocaine hydrochloride produced essentially no effect.

β-Cocaine was evaluated in parallel with cocaine in the guinea pig intradermal wheal test as described by Bülbring and Wajda.[6] The average threshold anesthetic concentration (TAC_5) was obtained from the dose-effect curve (semilogarithmic plot of duration in minutes *vs.* dosage) as described by Luduena and Hoppe.[8] Experimental values are furnished in Table I. β-Cocaine was approximately one-third as active as cocaine.

Acknowledgment. We thank Dr. Eugenio F. Bogado for the analgesic testing done on guinea pigs.

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Synthesis of Chlorozotocin, the 2-Chloroethyl Analog of the Anticancer Antibiotic Streptozotocin

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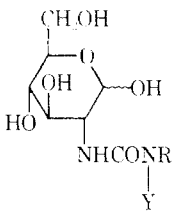
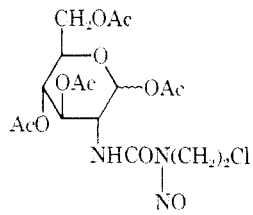
Streptozotocin (**1**) is a natural glucose-substituted *N*-methyl-*N*-nitrosourea,[1] a broad-spectrum antibiotic,[2] and an experimental anticancer agent[3] that has shown diabetogenic activity in animals[4] and clinical activity in the treatment of malignant insulinomas in man.[5] Prior synthesis of congeners of **1** has emphasized variation of the glucose moiety,[6-9] which resulted in enhancement of activity against leukemia L1210 in some cases judged on a comparable basis;[9] limited testing of the ethyl and butyl analogs of **1** indicated inactivity.[3] A moderate increase in the antileukemic activity of **1** was observed when the diabetogenic activity was suppressed with nicotinamide.[3,10] The structure and properties of **1** suggested some time ago the replacement of the methyl group with a 2-chloroethyl group, a modification demonstrated[11] to enhance markedly the effectiveness of a number of *N*-methyl-*N*-nitrosoureas against leukemia L1210.

The principal previous attempt to synthesize 2-[3-(2-chloroethyl)-3-nitrosoureido]-2-deoxy-D-glucopyranose (**3**, "chlorozotocin") involved deacetylation of the tetraacetate **4**,[12] a method modeled after the original synthesis of **1**[1] which has since been superseded by other methods.[13,14] Interest in the synthesis of **3** was recently revived in view of the observation that, in mice, the tetraacetate **4**, like **1**,[15] showed reduced bone-marrow toxicity and was, unlike **1**, nondiabetogenic.[16] Myelosuppression has been reported to be the limiting toxicity in the clinical use of the *N*-(2-chloroethyl)-*N*-nitrosoureas BCNU[17] [*N*,*N*′-bis(2-chloroethyl)-*N*-nitrosourea] and CCNU[18] [*N*-(2-chloroethyl)-*N*′-cyclohexyl-*N*-nitrosourea, **5a**].



1. R = Me; Y = NO
2. R = $(CH_2)_2Cl$; Y = H
3. R = $(CH_2)_2Cl$; Y = NO



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Chemistry. The (2-chloroethyl)urea **2** required for the synthesis of **3** was prepared by the addition of 2-chloroethyl isocyanate to D-glucosamine in water and later in anhydrous *N*,*N*-dimethylformamide (DMF). The latter yield-improving variation was tried as a model for similar conversions in which hydrolysis of the isocyanate would be a significant competitive reaction in an aqueous system. The liberation of D-glucosamine from its hydrochloride with a basic ion-exchange resin was also a convenient innovation.

The nitrosation of **2** with dinitrogen trioxide (chosen, as in the preparation of **1**,[14] to simplify the separation of water-soluble **3**) failed in a number of attempts with conventional media: (1) in formic acid, which would direct nitrosation to the desired position,[11] unwanted formylation (of presumably the primary hydroxyl group and possibly others) occurred; (2) in water, the preferred medium for the preparation of **1**,[14] and in dilute formic acid, the reaction was exceedingly slow and incomplete after several hours; (3) in dilute hydrochloric acid, the reaction was somewhat faster but gave at least two products as indicated by tlc. In concentrated hydrochloric acid, the reaction was essentially complete in less than 1 hr; recrystallization of the resulting precipitate produced an analytically pure sample of **3**. The pmr spectrum showed no evidence of random nitrosation and indicated a predominance of the α anomer. In aqueous solution, however, anomers of **3** would be expected to equilibrate as do various lots of crystalline **1**.[16] In a typical subsequent run, dinitrogen trioxide was introduced at a moderately fast rate until the reaction solution became red and intermittently thereafter until an appreciable amount of **3** had precipitated; precipitation was completed by the addition of a proportionately large volume of ether. Unrecrystallized **3** was suitable (melting point, ir, and tlc) for biological testing.

The enhanced rate of nitrosation in concentrated hydrochloric acid was attributed to *in situ* generation of nitrosyl chloride in analogy to the standard preparation involving the addition of a concentrated aqueous solution of sodium nitrite to concentrated hydrochloric acid.[19] The observed selective nitrosation was surpising, however, until the nitrosation of *N*-(2-chloroethyl)-*N*′-(*trans*-4-methylcyclohexyl)urea (**6**) under the same conditions (except that ethanol was added for solubilization) was demonstrated to give a nitrosourea that was identical with homogeneous samples of MeCCNU (**7**). Furthermore, a 1-hr treatment of a 2:1 mixture of **5a** and isomeric **5b** with ethanolic concentrated hydrochloric acid resulted in a high-yield transformation to a nitrosourea that was very nearly identical with homogeneous samples of CCNU. These results parallel the selective nitrosations and nitroso group migrations previous-