imide and 272 mg (1.32 mmol) of DCC in 4.5 mL of DMF. The reaction mixture was cooled, the DCU was removed by filtration, and 882 mg (1.32 mmol) of Arg-Gly-NH_2·dipicrate and 0.15 mL (1.35 mmol) of *N*-methylmorpholine were added. After standing at 25 °C for 6 days, the reaction mixture was added to 100 mL of cold, 5% $KHSO_4$ to give a gummy precipitate. The yellow aqueous layer was decanted and the gum dried. In order to obtain pure **1b**, extensive losses were taken. After CCD (240 transfers), tubes 100–130 containing product homogeneous by TLC [R_f (A) 0.16] were pooled and the solvent was evaporated. During CCD, picric acid was exchanged for HOAc on the guanidyl group of Arg. The addition of ether gave 39.2 mg (2.6% yield from H-Phe-Gln-Asn-Cys(Trt)-Pro·HCl) of **1b** as a fluffy powder: $[\alpha]^{25}_D$ −30° (*c* 1, HOAc). Anal. ($C_{49}H_{69}N_{15}O_{13}S_2 \cdot CH_3CO_2H \cdot 6H_2O$) C, H, N. Amino acid analysis: $^1/_2$-Cys, 1.92; Tyr, 0.37;[22] Phe, 1.00; Glu, 1.01; Asp, 1.01; Pro, 0.95; Arg, 1.07; Gly, 1.04; NH_3, 3.3.

Boc-Tyr(Me)-OCP (3). To a suspension of 19.4 g (99.0 mmol) of Tyr(Me) in 100 mL of 50% aqueous dioxane, 25 mL of 4 N NaOH was added in portions until solution was complete. Then, maintaining a constant pH of 10.5, 15.7 g (110 mmol) of Boc-N_3 was added, and the reaction was stirred at 25 °C for 6 h. At this point, the reaction mixture was extracted with ether to remove excess azide and acidified to pH 3 with 125 mL of 2 M citric acid, whereupon the product formed as a yellow oil. The mixture was extracted twice with 100-mL portions of EtOAc. The EtOAc solution was washed twice with water and then with 5 M NaCl and dried over anhydrous $MgSO_4$. The solvent was evaporated at reduced pressure to leave an approximately quantitative yield of product as a gum: TLC R_f (A) 0.78.

A solution of the oily Boc-Tyr(Me)-OH and 20.7 g (105 mmol) of 2,4,5-trichlorophenol in 150 mL of EtOAc was cooled to 4 °C and then a solution of 21.6 g (105 mmol) of DCC in 50 mL of EtOAc was added. After the reaction mixture stirred in an ice bath for 3 h, the DCU was removed by filtration and the EtOAc evaporated to leave a tan solid. Crystallization from ethanol yielded 27.4 g (58.4%) of active ester: TLC R_f (C) 0.85; mp 111–112 °C; $[\alpha]^{27}_D$ −25° (*c* 0.5, MeOH). Anal. ($C_{21}H_{22}Cl_3NO_5$) C, H, N, Cl.

Boc-Tyr(Me)-Phe-Gln-Asn-Cys(Trt)-Pro (4). A solution of 5.55 g (6.14 mmol) of H-Phe-Gln-Asn-Cys(Trt)-Pro·HCl·H_2O, 3.21 g (6.75 mmol) of **3**, and 1.4 mL (12.6 mmol) of *N*-methylmorpholine in 32 mL of DMF was stirred at 25 °C for 48 h, after which 0.2 mL (1.8 mmol) of $(CH_3)_2NCH_2CH_2NH_2$ was added to decompose excess active ester. The cooled (4 °C) reaction mixture was added to 300 mL of cold 1 N HCl to give a precipitate, which was filtered, washed with water, and dried in vacuo at 50 °C. The 2,4,5-trichlorophenol was removed by trituration with ether several times and the product dried again in vacuo for 3 h: yield 86.6%; TLC R_f (A) 0.70. Anal. ($C_{60}H_{70}N_8O_{12}S \cdot 2.5H_2O$) C, H, N, S.

Ac-Cys(Trt)-ONSu (5). A solution of 37.9 g (93.5 mmol) of Ac-Cys(Trt)-OH[23] and 10.1 mL of *N*-methylmorpholine in 450 mL of EtOAc was cooled to −15 °C. Maintaining this temperature, 12.2 g (92.5 mmol) of isobutyl chloroformate was added with vigorous stirring. After 30 s, 11.5 g (100 mmol) of *N*-hydroxysuccinimide was added, the mixture was filtered, and the filtrate was evaporated to a gum. Extraction of the gum with ether removed the active ester from byproducts, and the ether was evaporated in air to give 34.5 g (73.4% yield) of **5**: mp 184–186 °C; TLC R_f (A) 0.91; $[\alpha]^{25}_D$ +2.0° (*c* 1, HOAc), +7.3° (*c* 1, $CHCl_3$). Anal. ($C_{28}H_{26}N_2O_5S$) C, H, N, S.

H-Tyr(Me)-Phe-Gln-Asn-Cys(Trt)-Pro·HCl (6). To a solution of 15.8 g (14.1 mmol) of 4 in 50 mL of glacial acetic acid was added 25 mL of 5.93 N HCl/dioxane. After the solution was left standing at 25 °C for 15 min, the solvent and excess HCl were removed in vacuo at 40 °C. The product was solidified by trituration with ether, filtered, and dried in vacuo (4 h, 65 °C) to give a quantitative yield of **6** containing 1.25 mol of bound HCl: TLC R_f (B) 0.53; $[\alpha]^{25}_D$ −15.5° (*c* 1, MeOH). Anal. ($C_{55}H_{62}N_8O_{19}S \cdot 1.25HCl \cdot 3H_2O$) C, H, N, S, Cl.

Ac-Cys(Trt)-Tyr(Me)-Phe-Gln-Asn-Cys(Trt)-Pro (7). This compound was prepared in an identical manner to that of 4 from 3.70 g (3.31 mmol) of **6** and 1.91 g (3.81 mmol) of **5**. After purification by CCD (400 transfers, K = 9), a 62.0% yield was obtained: TLC R_f (B) 0.86; $[\alpha]^{25}_D$ −18.5° (*c* 1, MeOH). Anal. ($C_{79}H_{83}N_9O_{12}S_2 \cdot 3.5H_2O$) C, H, N, S.

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14-(Arylhydroxyamino)codeinones and Derivatives as Analgetics and Antagonists

Linda S. Schwab[1]

Department of Chemistry, University of Rochester, Rochester, New York 14627. *Received December 26, 1979*

Diels–Alder reaction of thebaine (1) and *N*-(cyclopropylmethyl)northebaine with nitrosobenzene and *p*-fluoronitrosobenzene gave adducts [6,14-*exo*-(phenyloxyamino)codeine 6-methyl ether and derivatives, **2a–d**] which yielded 14-(phenylhydroxyamino)codeinone and derivatives (**3a–d**) on acid hydrolysis. Rearrangement of **3** (NaOMe) afforded 5,14-*exo*-(phenyloxyamino)thebaimone and derivatives (**4a–d**); reduction of **3** led to 14-(phenylamino)dihydrocodeinone and derivatives (**5a–d**). Thebaine also reacted with 1-halo-1-nitrosocyclohexane (halo = Cl, Br) and with benzohydroxamic acid under oxidizing conditions to give 14-(hydroxyamino)codeinone (**6**). All compounds, **3–6**, were evaluated as analgetics and antagonists by the tail-flick, writhing, and Straub tail assays: compounds of types **3–5** were analgetics (*N*-Me), one-third to one-tenth as potent as morphine, or antagonists [*N*-(cyclopropylmethyl)], 50 to 100 times less potent than naloxone; **6** behaved as an antagonist in the tail-flick test but as an agonist in the other assays. Opiate receptor binding studies indicate that compounds of type **3** may be useful as opiate spin-label precursors.

The Diels–Alder reaction of thebaine (1, R = CH_3) with nitrosobenzene[2] or various postulated *C*-nitroso reactive intermediates[3–7] yields 1,2-oxazines (**2**) which hydrolyze in acid to 14-(hydroxyamino)codeinone derivatives.[2–4,6]

(1) Corresponding address: Center for Brain Research, University of Rochester Medical Center, Rochester, NY 14642.

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Table I. Physical Properties of the 14-Substituted Codeinones 2-5

no.	mp, °C	solvent	yield %	formula	anal.
2a	137-140[a]	EtOH/EtOAc	96	$C_{25}H_{26}N_2O_4$	C, H, N
2b	133.5-136	EtOAc	71	$C_{25}H_{25}FN_2O_4$	C, H, N
2c	123-125	MeOH	90	$C_{28}H_{30}N_2O_4$	C, H, N
2d	134-136	EtOAc	57	$C_{28}H_{29}FN_2O_4$	C, H, N
3a	128-131[b]		55		
3a·HCl	dec 135	acetone/MeOH	91	$C_{24}H_{24}N_2O_4 \cdot HCl$[c]	Cl
3b	126-128		59	$C_{24}H_{23}FN_2O_4$	C, H, N
3b·HCl	210-214 dec	acetone/MeOH	86		
3c·HCl	dec 150	acetone/MeOH	89	$C_{27}H_{28}N_2O_4 \cdot HCl$	C, H, N
3d·HCl	dec 160	acetone/MeOH	88	$C_{27}H_{27}FN_2O_4 \cdot HCl \cdot H_2O$	C, H, N
4a	209-210[d]	MeOH/EtOAc	56	$C_{24}H_{24}N_2O_4$	C, H, N
4b	182-183.5	EtOAc	41	$C_{24}H_{23}FN_2O_4$	C, H, N
4c	179-181	EtOH	68	$C_{27}H_{28}N_2O_4$	C, H, N
4d	dec 121	EtOH	22	$C_{27}H_{27}FN_2O_4 \cdot 0.5H_2O$	C, H, N
5a	189-192[e]	MeOH/EtOAc	57		
5b	237-239	EtOAc/C_6H_6	22	$C_{24}H_{25}FN_2O_3$	C, H, N
5c	137.5-139.5	EtOH	40	$C_{27}H_{30}N_2O_3$	C, H, N
5d	143-144	EtOH	32	$C_{27}H_{29}FN_2O_3 \cdot 0.5H_2O$	C, H, N

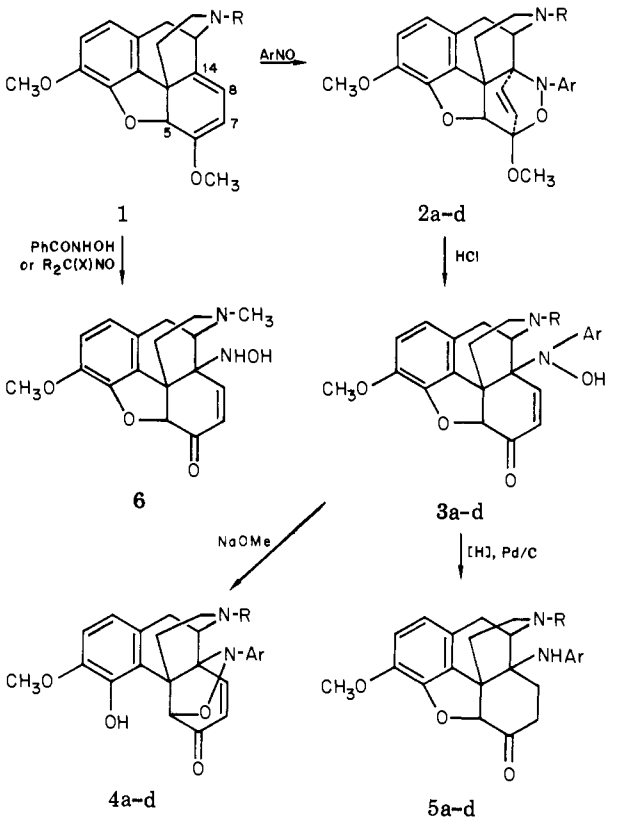
[a] Lit.[2] 115-118 °C. [b] Lit.[2] 127-128 °C. [c] Reported as the dihydrochloride.[2] [d] Lit.[2] 197 °C. [e] Lit.[2] 189-190 °C.

Arylhydroxyamines (**3**) so obtained reportedly yield analgetic 14-(arylamino)codeinones (**5**) on reduction;[2] the pharmacology of this class and its derivatives [including *N*-(cyclopropylmethyl) compounds as potential antagonists] merits further study. Furthermore, should compounds of type **3** bind to opiate receptors, oxidation to the corresponding nitroxide may afford novel codeinone spin-labels.

Chemistry. The synthetic sequence (Scheme I) followed the route used by Bentley and Kirby[2] for the compounds here designated the **a** series, but results in this laboratory differed at several points from the earlier work. These differences, as well as the properties of the novel **b**, **c**, and **d** series, are summarized in Table I. The stabilizing effect of a para substituent on aryl nitroxides suggested the inclusion of a para-substituted nitrosobenzene as dienophile. Attempted isolation and purification of *p*-(trifluoromethyl)nitrosobenzene gave mostly the azoxy dimer; *p*-nitrosoanisole did not form the adduct, but *p*-fluoronitrosobenzene reacted almost as readily with thebaine as did nitrosobenzene. The hydroxylamines **3** readily rearrange to the isomers **4** not only in the presence of NaOMe but also during recrystallization from ethanol, column chromatography on neutral alumina or silica gel, and reduction to the amines **5**; their hydrochlorides may be recrystallized and reduced without rearrangement. All the free bases **3**–**6** may be identified by the resonances of the C(5), C(7), and C(8) protons in the NMR spectrum; in addition, mass spectrometry differentiates the isomers **3** and **4** (see Experimental Section). The pale yellow color of **3a**,**b** suggested that some nitroxide might already be present, and, indeed, **3a** proved weakly paramagnetic; brief in situ oxidation with PbO_2 increased the concentration of nitroxide dramatically,[8] but the pure nitroxide has so far resisted isolation.

Dienes also react with *gem*-halogenonitrosoalkanes to give N-unsubstituted oxazines, which undergo reductive cleavage to 1,4-amino alcohols.[9] In contrast, attempted

Scheme I[a]



[a] a, R = Me, Ar = Ph; b, R = Me, Ar = *p*-F-Ph; c, R = cyclopropylmethyl, Ar = Ph; **d**, R = cyclopropylmethyl, Ar = *p*-F-Ph.

reduction of the *N*-aryloxazine **2a** with zinc/acetic acid or $LiAlH_4$ gave only thebaine, and catalytic hydrogenation yielded dihydrocodeine 6-methyl ether. Furthermore, although the reaction of thebaine with 1-chloro-1-nitrosocyclohexane appeared to be a convenient route to the series prototype **6**,[4] neither this reaction nor that using 1-bromo-1-nitrosocyclohexane succeeded consistently; 2-chloro- and 2-bromo-2-nitrosopropane failed to react at all. The reference sample of **6** was prepared by the reaction of thebaine with benzohydroxamic acid under oxidizing conditions,[4] but the unreliability of both this and the

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Table II. Analgetic and Antagonist Activities of Compounds 3-6 in Vivo and in Vitro

compd	tail flick test[a] test for	tail flick test[a] significance	tail flick test[a] max % analgesia	ED_{50}, mg/kg sc analgesia (writhing test)	ED_{50}, mg/kg sc antagonism (Straub tail)	opiate receptor binding, % control [^{3}H]DHM bound, no Na^+	opiate receptor binding, % control [^{3}H]naloxone bound, Na^+
3a	analgesia	–	20 (2-47)	1.45 (0.65-2.54)	>20	40	7
3b	analgesia	–	30 (7-65)	0.71 (0.44-1.04)	>40	45	14
3c	antagonism	+	0 (0-32)	>40	~7	34	60
3d	antagonism	+	0 (0-32)	~40	12 (9.2-15.7)	32	58
4a	analgesia	–	50 (18-82)	1.87 (1.54-2.29)	>40	38	-6
4b	analgesia	+	90 (56-99)	2.53 (0.39-5.78)	>40	44	2
4c	antagonism	inc.	20 (3-62)	>40	>40	5	52
4d	antagonism	inc.	20 (2-47)	~31	>40	21	53
5a	analgesia	–	10 (2-44)	1.04 (0.75-1.42)	>40	25	-3
5b	analgesia	–	10 (2-44)	0.76 (0.48-1.16)	>40	38	1
5c	antagonism	+	10 (2-44)	~40	~10.5	22	65
5d	antagonism	+	0 (0-32)	~25	~5	32	73
6	analgesia	–	10 (2-44)	5.0 (3.4-7.0)	>20	48	22
	antagonism	+	20 (2-47)				
M	analgesia	+	80 (39-97)	0.26 (0.18-0.40)	inactive	86	75
N	antagonism	+	0 (0-32)	inactive	0.09 (0.02-0.17)	72	89

[a] See Experimental Section for methods and statistical procedures. All compounds administered at 20 mg/kg except for M (morphine·H_2SO_4, 10 mg/kg) and N (naloxone·HCl, 5 mg/kg). Significance (Fisher test) defined as follows: for analgetics, – = $p > 0.05$ vs. vehicle (V) and + = $p \leq 0.05$ vs. V; for antagonists, inc. = $p \leq 0.05$ vs. V and M, and + = $p > 0.05$ vs. V and $p \leq 0.05$ vs. M.

preceding reactions halted the synthesis of 14-aminocodeinone at the hydroxylamine step.

Biological Results. The modified mouse tail-flick test of Ben-Bassat[10] provided a preliminary evaluation of compounds **3–6**. Table II shows the maximum percent analgesia (defined under Experimental Section) at 20 mg/kg for the *N*-methyl compounds and at 20 mg/kg followed by 10 mg/kg morphine for the *N*-(cyclopropylmethyl) compounds and **6**. Analgesia and antagonism were also judged by the Fisher exact probability test (Table II), which showed that only **4b** differed significantly from vehicle over the 2-h span of the experiment, but that all those tested as antagonists significantly blocked morphine analgesia.

The ED_{50} values for analgesia (writhing test) and antagonism (oxymorphinone Straub tail test) agree with this outline (Table II). The *N*-methyl compounds are from one-third to one-tenth as potent as morphine and show no antagonist activity; the *N*-cyclopropylmethyl series include antagonists 50 to 100 times less active than naloxone (**3c,d** and **5c,d**) and the apparently inactive **4c** and **4d**. In the opiate receptor binding assay, addition of Na^+ decreased the binding of the **a** and **b** series and increased that of the **c** and **d** series, discriminating between the former as agonists and the latter as antagonists.[11]

The unsubstituted hydroxylamine **6** reveals an unexpected pharmacological profile. Though more closely analogous to the potent agonist 14-hydroxycodeinone than the other *N*-methyl compounds tested, it is less active than any in the writhing assay. In the tail-flick test, instead of exerting the calming effect on the animals noted with **3a**, **3b**, **5a**, and **5b** (**3a** and **5a** also had a mild tranquilizing effect on rats), **6** appeared to increase the usual squirming and chewing behavior of the mice. Although inactive as an antagonist at 20 mg/kg in the Straub tail assay, **6** completely reversed the effect of morphine on the tail-flick response (Table II). The receptor binding assays defined **6** as an agonist.

Conclusions

Although several of the *N*-methyl compounds tested are moderately active analgetics, their inability to antagonize oxymorphinone signals physical-dependence-inducing potential. Among the *N*-(cyclopropylmethyl) compounds, **5c** and **5d** evince slight agonist activity, but all those tested are much less active than naloxone as antagonists. The unusual antagonist-like and excitatory activity of **6** observed in the tail-flick test may be the most interesting biological result in this series. In comparison, *N*-methylbenzazocines bearing an 11-(alkyl ethyl ketone) substituent (corresponding to the 14 position in morphine derivatives) are antagonists if the alkyl group comprises five or six carbons;[12] **6** shares with these *N*-methyl antagonists, as well as with buprenorphine, an activity profile marked by agonist activity in the writhing assay but not the tail-flick test. The results of the opiate binding assays for the arylhydroxylamines **3** indicate that, despite modest pharmacological activity, these compounds show promise as precursors for opiate receptor spin-labels.

Experimental Section

Melting points were taken on a Mel-Temp apparatus and are uncorrected. IR spectra were taken on a Perkin-Elmer 137 in Nujol mull, NMR spectra on a JEOL MH-100 in $CDCl_3$ with Me_4Si as internal standard, and mass spectra (MS) on a Dupont 490B spectrometer at 70 meV. Dr. Lewis Albro (Rensselaer, NY) and Chemalytics (Temp, AZ) performed the analyses.

Starting Materials. Northebaine hydrochloride was prepared from thebaine (Merck, Sharp and Dohme) in 88% yield by a modification of the diethyl azodicarboxylate (DAD) demethylation.[13] A solution of 25 mL of DAD in 50 mL of MeCN was added over 0.5 h to a stirred, refluxing solution of 47 g of thebaine in 250 mL of MeCN. Refluxing was continued an additional 1.5 h; pyridine hydrochloride (30 g) was added and the mixture allowed to cool to room temperature. The solid was collected, washed with MeOH, and dried at room temperature; concentration of the mother liquor under reduced pressure and trituration of

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the residue with MeOH gave a second and third crop. The crude northebaine hydrochloride (44 g, mp 265–270 °C, lit.[12] mp 270–272 °C) was used without further purification for reaction with cyclopropylcarbonyl chloride, followed by $LiAlH_4$ reduction of the amide to give *N*-(cyclopropylmethyl)northebaine.[14]

Caro's acid oxidation of *p*-fluoroaniline followed the procedure of Mijs[9] and required 0.5 h; the resulting *p*-fluoronitrosobenzene[15] (17% yield) was purified by column chromatography on silica gel (Baker, 40–140 mesh) with Et_2O. The *gem*-chloronitrosocyclohexane obtained by chlorination of cyclohexanone oxime in Et_2O at 0 °C using 5% aqueous NaOCl ("Clorox")[16] was purified similarly with CH_2Cl_2 as eluent; *gem*-bromonitrosocyclohexane (bromination in aqueous pyridine)[17] was used without purification.

Preparation of the Diels–Alder Adducts 2. Thebaine (6.2 g, 0.02 mol) and nitrosobenzene (2.2 g, 0.02 mol) were dissolved in 25 mL of CH_2Cl_2 and shaken until the green solution became olive (ca. 5 min); the solvent was removed under reduced pressure and the residue triturated with hot EtOH. The solid was collected after cooling and dried at room temperature overnight, affording **2a**; Table I includes further experimental details. The other adducts were prepared in the same way: **2b** from thebaine and *p*-fluoronitrosobenzene, **2c** from *N*-(cyclopropylmethyl)northebaine and nitrosobenzene, and **2d** from *N*-(cyclopropylmethyl)northebaine and *p*-fluoronitrosobenzene. The NMR spectra of all the adducts were characterized by δ 4.67–4.77 [s, C_5 H] and 5.26–5.35 and 6.23–6.29 [d, 1 H each, J = 9 Hz, $C_{7,8}$ H]; MS showed only the starting materials.

Hydrolysis of the Diels–Alder Adducts (3). The adduct **2a** (8.4 g, 0.02 mol) was added in 1-g portions to 85 mL of vigorously stirred 1 M HCl. The suspension was stirred for 3 h and filtered; the filter cake was washed with acetone and dried in a vacuum desiccator over P_2O_5 to give **3a**·HCl. The HCl salts of **3b**, **3c**, and **3d** were prepared in the same way and characterized by IR, 1680–1682 cm^{-1} (C=O), and by MS, M^+, $M^+ - 16$, and $M^+ - 18$.

The bases were freed by shaking the recrystallized salt with ca. 40 parts (w/v) each of saturated aqueous $NaHCO_3$ and CH_2Cl_2 until all solid dissolved (5 min); the organic phase was separated, dried (Na_2SO_4), and concentrated under reduced pressure. The free bases of **3a** and **3b** were purified by diluting the concentrated solution with EtOAc and cooling. The yellow crystals so obtained were filtered, dissolved again in CH_2Cl_2, concentrated until crystals reappeared, and diluted and isolated as before. The bases **3c** and **3d** were viscous oils; NMR spectra of the bases included δ 4.84–4.89 (s, C_5 H) and 5.92–5.96 and 6.29–6.33 (d, 1 H each, J = 10 Hz, $C_{7,8}$ H).

Rearrangement of the 14-(Arylhydroxyamino)codeinones (4). The hydroxylamine **3a** (0.25 g, 0.6 mmol) was added to a filtered solution of 0.05 g of NaOMe in 5 mL of MeOH, and the mixture heated until solution was complete; cooling gave **4a** as fine yellow needles. Similarly prepared were **4b**, **4c**, and **4d**. The products were characterized by IR, 1683–1689 cm^{-1} (C=O), by NMR, δ 5.12–5.15 (s, C_5 H), 5.71–5.8 (br s, ArOH) and 6.05–6.07 (s, 2 H, $C_{7,8}$ H), and by MS, M^+ and $M^+ - 29$, 30, or 31.

Reduction of the 14-(Arylhydroxyamino)codeinones (5). The recrystallized salt **3a**·HCl (1.50 g, 3.4 mmol) was dissolved in 150 mL of MeOH; 0.75 g of 10% Pd/C (Matheson, Coleman and Bell) was added, and the mixture was hydrogenated for 2.5 h at 56 psig on a Parr hydrogenation apparatus. The catalyst was removed by filtration and the solvent evaporated under reduced pressure. The residual gum was shaken with equal volumes (100 mL) of saturated aqueous $NaHCO_3$ and CH_2Cl_2; the organic phase was separated and dried (Na_2SO_4) and the solvent removed under reduced pressure. Recrystallization gave white needles of **5a**. The aminocodeinones **5b**, **5c**, and **5d** were prepared in the same way and were identified by IR, 1725–1735 cm^{-1} (C=O), by NMR, δ 4.64–4.67 (s, C_5 H), and by M^+ in the mass spectrum.

14-(Hydroxyamino)codeinone Hydrochloride (6). Method A. Thebaine (4.7 g, 15 mmol) in 20 mL of CH_2Cl_2 was allowed to react with benzohydroxamic acid in the presence of tetraethylammonium periodate[18] as described by Kirby and Sweeny.[3] After the organic phase was separated, washed with water, and dried (Na_2SO_4) and the solvent removed under reduced pressure, the syrupy orange residue was dissolved in 10 mL of hot MeOH. The mixture was cooled to room temperature and 3 M HCl was added dropwise until acid to pH paper. After 15 min, the solvent was removed under reduced pressure and the residue was triturated with acetone containing a few drops of MeOH, yielding 2.6 g (47%) of **6**·HCl; the analytical and test samples were purified by recrystallization from acetone/MeOH: mp 244.5–246 °C dec; MS *m/e* 328 (M^+), 312, 311, 297; IR 3215 (OH), 1678 cm^{-1} (C=O). Anal. ($C_{18}H_{20}N_2O_4$·HCl) C, H, N.

The free base was prepared in the same way as **3**. The resulting white foam was triturated with petroleum ether (30–60 °C) to give a 50% yield of a noncrystalline tan powder: dec above 100 °C; IR 3205 and 1675 cm^{-1}; NMR δ 2.39 (s, 3 H, NCH_3), 3.85 (s, 3 H, OCH_3), 4.88 (s, 1 H, C_5 H), 6.28 (q, J = 10 Hz, 2 H, $C_{7,8}$ H) 6.65 (q, J = 9 Hz, 2 H, $C_{1,2}$ H).

Method B. Thebaine (0.16 g, 0.5 mmol) was allowed to react with *gem*-bromonitrosocyclohexane (0.4 g, 2 mmol) in $CHCl_3$/EtOH/H_2O (3:8:3 mL) for 16 h at room temperature. The solvents were removed under reduced pressure; the residue was heated with 1:1 EtOH/EtOAc and cooled, affording 0.06 g (30%) of **6**·HBr, identified as the free base by MS and NMR in comparison with material from method A.

Method C. The reaction of thebaine with *gem*-chloronitrosocyclohexane (1:4 molar ratio) in CH_2Cl_2/EtOH/H_2O (1:2:1) was carried out as in the preceding method to give 60% **6**·HCl, mp 250–254 °C dec, whose free base exhibited NMR and mass spectra identical with those from material obtained by method A.

Pharmacological Methods. Young male Swiss white mice (Charles River Breeding Laboratories, Wilmington, MA) weighing ca. 35 g were used in the tail-flick test.[10] The mice were restrained in tagboard cylinders; hot water (54 °C) provided the nociceptive stimulus, and the pain reaction time (PRT; the shorter of each pair of measurements) was recorded before injection and at 15-min intervals thereafter up to 2 h. Naive animals not responding under 2.2 s were removed from the experiment; 5 s was the maximum exposure time for injected animals. Test compounds were administered ip at 20 mg/kg as propylene glycol suspensions (**3a,b**, **4a–d**, and **5a–d**) or aqueous solutions (morphine sulfate, naloxone hydrochloride, **3c**·HCl, **3d**·HCl and **6**·HCl) to a group of 9–11 animals; challenge with 10 mg/kg morphine followed immediately for compounds tested as antagonists. The cumulative mean control PRTs with their standard deviations were 1.3 ± 0.4 (saline) and 1.4 ± 0.4 s (propylene glycol); hence, analgesia was defined as a PRT more than 3 standard deviations greater than the control mean, i.e., 2.6 s. Percent analgesia is therefore the percent of animals at a given time in a state of analgesia; maximum percent analgesia (Table II) is the largest such value observed at any single time within the 2-h test. Confidence limits (95%) were determined as in ref 19a,b. The Fisher exact probability test[19c] provided another measure of analgesia: the mean PRT was determined for each mouse in a test group over the 2-h experiment and these values cast against those for vehicle, and among putative antagonists, against those for morphine as well. Cited references describe the phenylquinone writhing assay[20] and the oxymorphinone Straub tail test for antagonism.[21] The opiate receptor

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binding assay employed a standard method,[22] in triplicate, using [^{3}H]dihydromorphine or [^{3}H]naloxone; in the latter case, the medium was 0.1 M in NaCl.

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New Anticonvulsants: Schiff Bases of γ-Aminobutyric Acid and γ-Aminobutyramide

Jean-Pierre Kaplan,* Bernard M. Raizon,

Department of Chemistry, CNS Unit, Laboratoires d'Etudes et de Recherches SYNTHELABO, 92220 Bagneux, France

Michel Desarmenien, Paul Feltz, P. Max Headley,

Laboratoire de Physiologie des Centres Nerveux, Université Pierre et Marie Curie, 75230 Paris, France

Paul Worms, Kenneth G. Lloyd,

Department of Biology, CNS Pharmacology Unit, Laboratoires d'Etudes et de Recherches SYNTHELABO, 92220 Bagneux, France

and Giuseppe Bartholini

Research Department, Laboratoires d'Etudes et de Recherches SYNTHELABO, 75013 Paris, France. *Received July 25, 1979*

Schiff bases of γ-aminobutyric acid (γAbu) and γ-aminobutyramide ($\gamma AbuNH_2$) were prepared and tested for anticonvulsant and γAbu mimetic activity. 4-[[(4-Chlorophenyl)(5-fluoro-2-hydroxyphenyl)methylene]amino]butanoic acid monosodium salt (4) and 4-[[(4-chlorophenyl)(5-fluoro-2-hydroxyphenyl)methylene]amino]butanamide (5) blocked bicuculline-induced lethality and convulsions and displaced [^{3}H]γAbu from its membrane binding sites. In the rat dorsal root sensory ganglion, compound 4 exhibited γAbu agonist properties. Compounds 4 and 5 are thus anticonvulsants and directly acting γAbu mimetics.

Under physiological conditions γ-aminobutyric acid (γAbu) poorly crosses the blood–brain barrier.[1] Of the putative γAbu mimetics prepared to date which enter the brain, only muscimol has been widely examined, and even this compound enters the brain to a very limited extent.[2] However, muscimol exhibits an unacceptable central nervous system toxicity, which prevents its wide clinical use.[3,4] From the pharmacological activities of muscimol and the present concepts on possible γAbu involvement in neuropsychiatric disorders, it is likely that a nontoxic γAbu mimetic which easily enters the brain will have useful therapeutic properties. With this in mind, we have prepared derivatives of γAbu with an imine link (Schiff base) to a lipophilic carrier[5-9] in order to facilitate the passage of γAbu across the blood–brain barrier.[10]

Table I. Acute Lethality and Antibicuculline Activities

compd	LD_{50},[a] mg/kg ip	LD_{50},[a] mg/kg po	ED_{50},[b] mg/kg ip lethality	ED_{50},[b] mg/kg ip convulsions
4	420 ± 40	>2000	70 ± 7	15 ± 4
5	900 ± 160	>3000	65 ± 8	20 ± 3
dipropyl-acetate[c]	1000 ± 50		130 ± 22	33 ± 4
diphenyl-hydantoin	250 ± 49		50 ± 13	12.5 ± 4
muscimol	17 ± 3		>2	0.25 ± 0.1

[a] LD_{50} refers to the dose which causes death in 50% of the mice within 48 h (ip) or 7 days (po). [b] ED_{50} refers to the dose which decreases by 50% the lethality or convulsions which are induced by bicuculline in mice. [c] Refers to the sodium salt.

Table II. Displacement of [^{3}H]γAbu Binding

compd	K_i, μM rat brain	K_i, μM human cerebellum
4	0.59 ± 0.08	0.68 ± 0.09
5	14 ± 3	40 ± 7
muscimol	0.004 ± 0.001	0.011 ± 0.002
γAbu	0.025 ± 0.001	0.20 ± 0.02
dipropylacetate[a]	>100	>100
diphenylhydantoin	>100	>100

[a] Refers to the sodium salt.

Chemistry. $\gamma AbuNH_2$ reacted smoothly with the benzophenone **2** obtained from a Fries rearrangement of the ester **1**, to give compound **5** in good yield (Scheme I). The presence of the hydroxyl group on the benzophenone facilitated the formation of the imine bond.[7,8] The acid **3** was similarly obtained and converted to the sodium salt **4**, in ethanol. The IR, UV, and NMR spectra of the com-

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