Use of the Butaclamol Template in a Search for Antipsychotic Agents with Lessened Side Effects[1a]

Michael J. Kukla,*[1b]

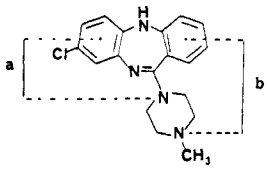
Department of Chemistry

James L. Bloss, and Linda R. Brougham

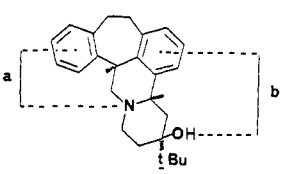
Department of Biology, G. D. Searle and Company, Chicago, Illinois 60680. *Received July 14, 1978*

A number of molecular similarities between the antipsychotic agents butaclamol and clozapine were noted. Based on the premise that this was a strong indicator of a common mechanism of action (i.e., binding at the antagonist state of the dopamine receptor), a research approach was described. Three simplified analogues (**4**, **8**, and **12a**) of butaclamol which still retained the molecular functionalities of the parent structure were synthesized and tested in the haloperidol receptor assay. 1-(5-Methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene)-4-*tert*-butyl-4-piperidine (**12a**) displaced tritiated haloperidol with an IC_{50} value of 2.4 nM, as compared to a value of 0.5 nM for butaclamol. However, when **12a** was tested in vivo or in the spiroperidol receptor assay it was found to be considerably less potent.

A need exists for antipsychotic agents which avoid the parkinsonian and tardive dyskinesias side effects. Clozapine is the only compound found to date which displays



CLOZAPINE



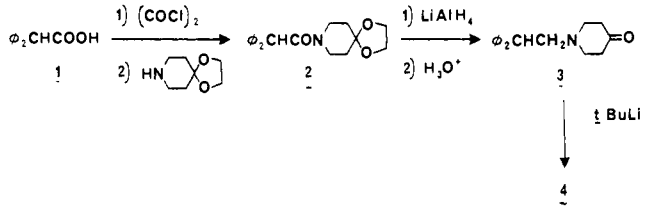
BUTACLAMOL

clinical efficacy with virtually no incidences of the aforementioned untoward symptoms.[2a,b] Unfortunately, both tachycardia[2c] and agranulocytosis[3] have been associated with its use.

Recently, butaclamol, a novel structural type, was shown to possess antipsychotic activity, but like most other drugs it was plagued with the extrapyramidal side effects.[4] However, its unique structure is interesting in a number of ways. This compound possesses four optical centers, and separation of the enantiomers led to the finding that only one displayed the desired effects. This aided greatly in defining stereospecific binding in the dopamine, haloperidol, and spiroperidol receptor assays.[5-12] The structural rigidity of this molecule makes it a virtual blueprint for definition of the structural requirements for binding at the neuroleptic receptors. This was borne out by its very high affinity for displacement of tritiated haloperidol and spiroperidol.

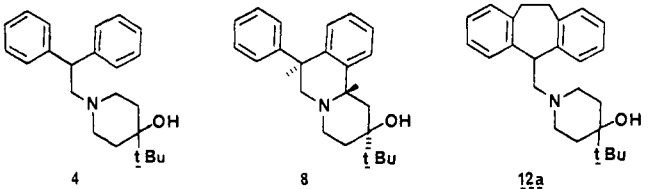
As displayed pictorially, there are some striking similarities between clozapine, itself a relatively rigid structure, and butaclamol.[13] The distance "*a*" between the center of a given phenyl ring and the proximate nitrogen approximates the extended phenethyl-amine distance of dopamine and is almost identical for the two molecules. The same can be said for "*b*", which denotes the distance between the other phenyl ring and a polar substituent (tertiary nitrogen in clozapine and an alcohol oxygen in butaclamol). Even more intriguing was the comparison between the two molecules of the relative orientation of the two imaginary lines traced by distances *a* and *b* in each compound. They are very similar. Thus, examination of Dreiding models revealed that the molecules can very nearly be overlayed one upon the other. The implication of this kind of analysis is that the drugs could be effective via the same mechanism of action, possibly by attachment at the dopamine receptor in its antagonist state. If this were true, one could be confident that modification of the butaclamol structure might lead to drugs which, like clozapine but unlike butaclamol, would be devoid of the side effects discussed earlier. In other words, a molecule could be an effective antipsychotic agent by virtue of dopamine receptor blockade yet escape the parkinsonism and/or tardive dyskinesias.

Scheme I



The strategy was relatively simple. The first step was to find a butaclamol analogue which maintained the structural features (phenyl rings and polar substituents) of the parent molecule, was more readily available synthetically, and retained the ability to bind at the dopamine–haloperidol receptor. The second step in the process was to make small changes in the simplified structure with the hope that ultimately a compound with cleaner antipsychotic activity would be discovered.

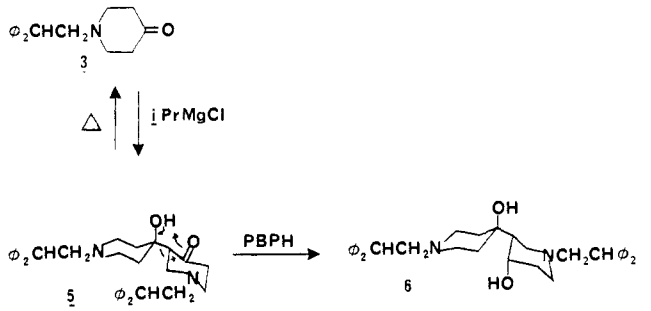
The following report describes the initial progress in this project. Three simplified analogues of butaclamol (**4**, **8**, and **12a**) have been synthesized, and some simple structural modifications have been carried out on **12a**, a compound which showed a significant haloperidol receptor binding.



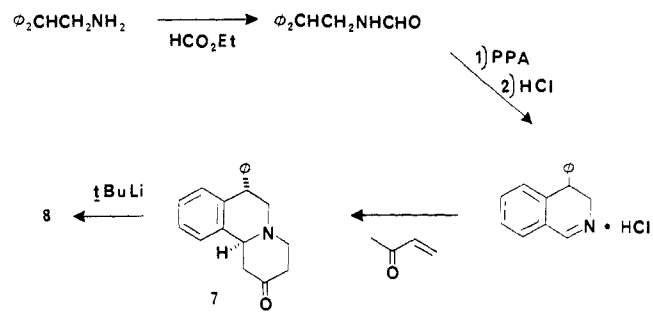
Chemistry. Synthesis of the most flexible analogue (**4**) of butaclamol is pictured in Scheme I. Diphenylacetic acid was converted to the acid chloride by treatment with oxalyl chloride. Subsequent reaction with 4-piperidinone ethylene ketal yielded the amine ketal **2**. Reduction of the carbonyl with lithium aluminum hydride resulted in a hygroscopic amino ketal which was hydrolyzed with 14% aqueous perchloric acid to give piperidone **3**. Addition of *tert*-butyllithium to the carbonyl led to the desired tertiary carbinol **4**. If the corresponding Grignard reagent was employed, as well as cyclohexyl- or isopropylmagnesium chloride, none of the desired addition products to **3** were formed. Rather, there was a clean transformation to the

0022-2623/79/1822-0401$01.00/0

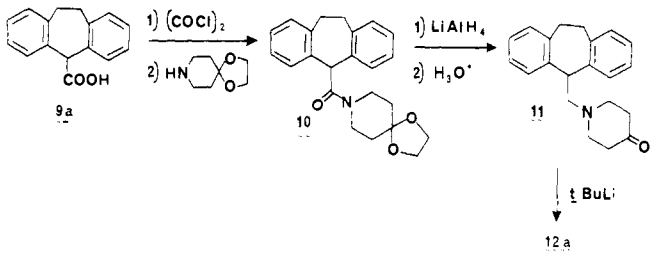
Scheme II



Scheme III



Scheme IV



dimer **5** (Scheme II) which resulted from an aldol condensation. This dimerization was thermally reversible, as witnessed by the fact that an attempt to sublime [150 °C (0.1 mm)] a sample of **5** gave only pure **3** on the cold finger. Reduction of the ketone **5** with the bulky reducing agent, lithium perhydro-9b-boraphenalylhydride, gave a single isomer which presumably was of the cis axial alcohol configuration (**6**).

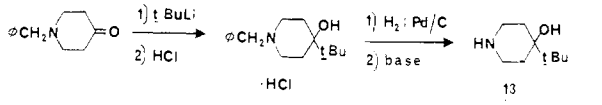
The benzo[*a*]quinolizinol derivative **8** was obtained from ketone **7**, which was synthesized by the published[14] series of steps outlined in Scheme III. The assigned stereochemistry of this molecule (**7**) and the alcohol **8** is based on analogy to that obtained in the synthesis of butaclamol.[16,17] We isolated only a single isomer of **8** which we assume corresponded to the predominant isomer of butaclamol found by the Ayerst group. This contrasts to the case of the methylcarbinol in which we obtained and isolated two stereoisomers.

The tricyclic compound **12a** was procurred via two related routes. The first (Scheme IV) involved the same sequence of transformations described above for the synthesis of **4**. An alternative is the converging route pictured in Scheme VI, in which 4-*tert*-butylpiperidin-4-ol (**13**)[15] (Scheme V) was reacted with the appropriate acid chloride. This was conducive to the synthesis of related structures. The amides **14a–f** obtained were resistant to reduction by lithium aluminum hydride, but aluminum hydride converted them smoothly to the amines **12a–f**.

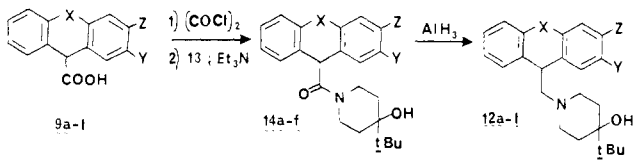
Results and Discussion

The simplified analogues of butaclamol **4**, **8**, and **12a** were synthesized. Since the initial premise revolved around binding at the haloperidol receptor as the possible common mechanism of action of butaclamol and clozapine, this in vitro assay was used to assess the potency of **4**, **8**, and **12a** as butaclamol mimics. As indicated in Table I, **4** and **8** were found to be somewhat less potent at displacing tritiated haloperidol, whereas **12a** was nearly equipotent to the parent structure in this regard. This was very surprising, since the former (**12a**) is a less rigid structure. Based on these data, the related structures **12b–f** were synthesized and tested in the same assays. Again there was significant binding in each case, although none bound as well as **12a**.

Scheme V



Scheme VI



	X	Z	Y
a	CH_2CH_2	H	H
b	CH=CH	H	H
c	CH_2CH_2	Cl	H
d	CH_2CH_2	H	Br
e	S	H	H
f	O	H	H

Table I. Displacement of [^{3}H]Dopamine (DA), [^{3}H]Haloperidol (Halo), and [^{3}H]Spiroperidol (Spiro)

compd	[^{3}H]DA[a]	[^{3}H]Halo[a]	[^{3}H]Spiro[a]
4	6.0×10^{-6}	1.6×10^{-7}	3.6×10^{-6}
5	2.7×10^{-5}	$5\text{-}10 \times 10^{-7}$	nt
6	2.5×10^{-5}	9.0×10^{-8}	nt
8	1.6×10^{-5}	3.0×10^{-8}	7.9×10^{-7}
12a	1.5×10^{-5}	2.4×10^{-9}	8.6×10^{-7}
12b HCl	7.2×10^{-6}	3.7×10^{-8}	1.5×10^{-6}
12c HCl	8.8×10^{-7}	7.5×10^{-9}	2.7×10^{-7}
12d HCl	1.7×10^{-6}	2.8×10^{-7}	2.2×10^{-7}
12e HCl	4.5×10^{-6}	2.9×10^{-7}	9.1×10^{-7}
12f HCl	1.5×10^{-5}	3.1×10^{-7}	5.9×10^{-6}
(+)-butaclamol	1.0×10^{-7}	5.0×10^{-10}	1.8×10^{-9}
clozapine	8.0×10^{-6}	1.8×10^{-7}	8.4×10^{-7}

[a] These are molar drug concentrations which inhibit specific binding of 2.5 nM [^{3}H]dopamine, 1.6 nM [^{3}H]haloperidol, and 0.15 nM [^{3}H]spiroperidol by 50% (i.e., IC_{50}). Nonspecific binding is measured in the presence of 10^{-5} M (+)-butaclamol for [^{3}H]DA and [^{3}H]Spiro or 10^{-4} M dopamine for [^{3}H]Halo. IC_{50} values were determined from log probit plots using four to six concentrations of each compound assayed in triplicate.

The results of the in vivo testing[20] on this group of compounds were very perplexing. Observations of the gross symptomatology in mice at doses of 10–320 mg/kg ip almost universally included depression, ataxia, chronic seizures, respiratory depression, and flacidity in common with butaclamol and clozapine. However, this was where the similarity ended. Table II catalogs the testing results and points out the differences between the test compounds and standards. Both butaclamol and clozapine displayed a relatively potent blockade of a conditioned avoidance response at a minimum effective dose of 0.5 and 10 mg/kg, respectively. Only **12a** was active in this procedure at a subtoxic dose.

Table II. In Vivo Testing Results

compd	conditn avoid. response[a]	amphetamine stereotypy[b] gnawing	licking	sniffing	amphetamine lethality[a]	LD_{50}, mg/kg
4	I (50)	20 (64)	20 (64)	0 (64)	I (50)	*d*
8	I (40)	38.8	48.1	44 (128)	I (20)	>160
12a	A (20)	33 (64)	33 (64)	66 (64)	A (20)	80
12b	A (40)	50 (16)	50 (16)	0 (16)	I (10)	39
12c	I (40)	34.5	39.2	0 (64)	I (64)	160-320
12d	I (40)	26.6	31.3	0 (64)	I (36)	180
12e	I (40)	10 (128)	0 (128)	0 (128)	I (64)	>320
12f	I (40)	10 (128)	10 (128)	0 (128)	I (64)	160-320
butaclamol	A (0.5)	0.05	0.07	0.76	I (32)	>320
clozapine	A (10)	59.2	46.9	98.4	2[c]	160

[a] Compounds are rated active (A) or inactive (I) and either the minimum effective dose (mg/kg) or highest inactive dose (mg/kg) tested, respectively, is presented in parentheses. [b] Below each type of stereotypic behavior induced by amphetamine (gnawing, licking, or sniffing) is given either the ED_{50} (mg/kg) for blockade of that behavior or in what percentage of animals that behavior was blocked at the dose (mg/kg) given in parentheses. [c] ED_{50}, mg/kg. [d] Undetermined. A dose of 64 mg/kg caused no lethalities.

In the drug-interaction tests employed, blockade of amphetamine-induced stereotypy and blockade of amphetamine lethality, there was a wide variance between the model compounds. Butaclamol was extremely potent in the former and relatively inactive in the latter, while clozapine had virtually the opposite pharmacological effects. None of the synthetic molecules approach the potency of butaclamol in its ability to inhibit any of the stereotypic behaviors, i.e., gnawing, licking, or sniffing. By the same token, all of the compounds, except **12a**, were rated inactive at protecting mice against a lethal dose of *d*-amphetamine.

As an overview, none of the compounds approached the in vivo potency, when compared to butaclamol, that one would expect based on their ability to displace labeled haloperidol, most notably **12a**. However, when these molecules were evaluated in the more recently reported spiroperidol assay[11,12] the IC_{50} values (Table I) obtained seem to better reflect the in vivo data.

The reported compounds were synthesized as simplified analogues of butaclamol because clozapine has strikingly similar molecular features, either coincidentally or significantly, to this rigid structure. Consequently, it is interesting to note that **12a** has both an in vitro and in vivo profile more similar to clozapine in the limited number of tests employed. Whether this simply reflects a lack of potency in **12a** compared to butaclamol or is a profound difference in activity is an issue to be explored in future work in this approach.

Experimental Section

Melting points were taken on a Thomas-Hoover apparatus and are uncorrected. Where analyses are indicated by symbols of the elements, the analytical results are within ±0.4% of the theoretical values. In the cases where more than one reaction was carried out by basically the same procedure, only a typical example is reported.

[G-^{3}H]Haloperidol, [1-*phenyl*-4-^{3}H]spiroperidol, and [*N*-*ethyl*-1-^{3}H]dopamine were purchased from New England Nuclear. Nonradioactive dopamine was purchased from Sigma Chemical Co. Clozapine and (+)-butaclamol were gifts from Sandoz Pharmaceuticals and Ayerst Laboratories, respectively.

2,2-Diphenylacetyl Chloride. Diphenylacetic acid (25 g) and oxalyl chloride (25 mL) were codissolved in 300 mL of benzene and heated under reflux for 40 h. The mixture was concentrated on a rotary evaporator. Two additional portions of benzene were added and subsequently distilled in vacuo to remove excess reagent. This yielded 29.2 g of clear brownish oil whose IR spectrum (C=O, 1795 cm^{-1}) was clearly that of the acid chloride. This was used without further purification.

1-(2,2-Diphenylacetyl)-4-piperidinone Ethylene Ketal (2). To a stirred solution of diphenylacetyl chloride (24 g, 0.1 mol) in 250 mL of benzene were added solid sodium carbonate (15 g) and 4-piperidinone ethylene ketal (16.4 g, 0.11 mol). The resultant mixture was heated under reflux for 29 h and allowed to sit overnight at room temperature before it was filtered. The filtrate was washed twice with 10% HCl (aqueous), twice with saturated sodium bicarbonate solution, and once with brine. It was dried with $MgSO_4$ and concentrated to yield 32.9 g (93.5%) of tan solid, which was used without further purification. A small 3-g sample was recrystallized from cyclohexane to yield 2.3 g of pale yellow crystals, mp 136–137 °C. Anal. ($C_{21}H_{23}NO_3$) C, H, N.

1-(2,2-Diphenylethyl)-4-piperidinone Ethylene Ketal. The amide **2** (35.4 g, 105 mmol) was dissolved in THF (300 mL) and added dropwise over 60 min to a mechanically stirred suspension of $LiAlH_4$ (8 g, 210 mmol) in THF (600 mL) under reflux and a nitrogen atmosphere. The resultant green mixture was heated an additional 42 h before it was cooled with an ice bath and quenched with the careful sequential addition of 8 mL of H_2O, 8 mL of 15% NaOH (aq), and 24 mL of H_2O. It was stirred for 3 h before the granular aluminum salts were removed by vacuum filtration and then washed thoroughly with ether. The filtrate was concentrated to yield 35.2 g of pale yellow oil, which crystallized on sitting. A small sample was recrystallized from cyclohexane but it was too hygroscopic to allow for elemental analysis. Thus, it was used without further purification.

1-(2,2-Diphenylethyl)-4-piperidinone (3). The ketal (33.2) was suspended in 1 L of 14% aqueous perchloric acid and heated on a steam bath for 24 h while being mechanically stirred. The mixture was allowed to cool to room temperature and basified with 50% NaOH (aq) before extraction with three portions of ethyl acetate (1 L total). The combined organic layers were dried with brine and $MgSO_4$ and concentrated to yield 27 g of oily yellow solid which was recrystallized from absolute ethanol to yield 11.5 g of tan solid: mp 140.5–141.5 °C; NMR ($CDCl_3$,Me_4Si) δ 2.3 (t, J = 6 Hz, 4 H, $-CH_2CO-$), 2.8 [t, J = 6 Hz, 4 H, $-N(CH_2CH_2)_2CO$], 3.1 (d, J = 8 Hz, 2 H, $-CH_2N$), 4.2 (t, J = 8 Hz, 1 H, methine), 7.2 (s, 10 H, aromatics). Anal. ($C_{19}H_{21}NO$) C, H, N; C: calcd, 81.68; found, 81.14.

1-(2,2-Diphenylethyl)-3-[1-(2,2-diphenylethyl)-4-hydroxy-4-piperidinyl]-4-piperidinone (5). To a stirred, cooled (ice bath) solution of ketone **3** (4.0 g, 14.4 mmol) in THF (50 mL) under nitrogen was added, over 2 min, isopropylmagnesium chloride in ether (9.8 mL of 2.2 M). The mixture was stirred at 0 °C for 75 min before it was quenched with the careful addition of 50 mL of 10% NH_4Cl (aq) solution. The organic layer was separated, and the aqueous phase was extracted with additional ether. The combined organic fractions were dried and concentrated to yield 4.5 g of yellow oil. This material was purified on silica gel with low-pressure liquid chromatography to yield 1.3 g of pale yellow glass upon elution with 10% acetone–CH_2Cl_2. Trituration with heptane gave a solid which was recrystallized from a minimal amount of 95% ethanol: mp 125–127 °C; IR ($CHCl_3$) 3310 (–OH) and 1695 cm^{-1} (C=O). Anal. ($C_{38}H_{42}N_2O_2$) C, H, N.

1-(2,2-Diphenyl)-4-*tert*-butyl-4-piperidinol (4). To a stirred solution of ketone **3** (2.0 g, 7.2 mmol) in benzene (50 mL) under nitrogen was added *tert*-butyllithium (30 mL of 1.6 M in pentane). The deep-red solution was stirred for 23 h before it was quenched

with the careful addition of 60 mL of 10% NH_4Cl (aq) solution. After 1 h, the organic phase was separated, washed with water and brine, dried with $MgSO_4$, and concentrated to yield 2.36 g of clear red oil. Chromatography on 200 g of E-Merck silica gel yielded a pale yellow solid upon elution with methylene chloride (750 mL), followed by 1% ethanol–methylene chloride (500 mL). Sublimation [130 °C (0.1 mm)] yielded 500 mg of white solid: mp 105–110 °C; NMR ($CDCl_3$, Me_4Si) δ 0.9 (s, 9 H, *tert*-butyl), 3.0 (d, J = 7 Hz, 2 H, $-CH_2N$), 4.2 (t, J = 7 Hz, 1 H, methine).

1-(2,2-Diphenylethyl)-3-[1-(2,2-diphenylethyl)-4-hydroxy-4-piperidinyl]-4-piperidinol (6). Lithium perhydro-9b-boraphenallylhydride (3.5 mL of 1.5 M in THF) was added via syringe to a stirred, cold (−78 °C) solution of ketone **5** (650 mg, 1.17 mmol) in THF (25 mL) under nitrogen. After 30 min, the reaction was quenched with the addition of 25 mL of 10% NH_4Cl (aq) solution. Ether was added and the mixture was allowed to warm to room temperature. After 2 h, the organic layer was separated and then extracted twice with 10% HCl (aq) solution. The combined acid extracts were basified with 50% NaOH (aq) solution and then extracted twice with ether. These organic fractions were combined, dried, and concentrated to yield 650 mg of yellow gum, which crystallized when triturated with ether. Recrystallization from a minimal amount of absolute ethanol yielded a white solid, mp 174–175 °C, which had no carbonyl band in the infrared. Anal. ($C_{38}H_{44}N_2O_2$) C, H, N.

2-*tert*-Butyl-1,3,4,6,7,11b-hexahydro-7-phenyl-2*H*-benzo[*a*]quinolizin-2-ol (8). Ketone **7** (4.2 g, 15.3 mmol) was dissolved in 100 mL of benzene, and to the stirred solution was added *tert*-butyllithium in pentane (66 mL of 1.39 M) under nitrogen. The reaction was monitored by thin-layer chromatography of quenched aliquots and, although there was only a single major product, a large amount of starting material remained. This could reflect anion formation of the starting ketone, which would be inert to nucleophilic addition. The dark mixture was stirred for 29 h before it was cooled with an ice bath and quenched with the addition of 25 mL of aqueous 10% NH_4Cl solution. The organic layer was separated, washed with water and brine, dried with $MgSO_4$, and concentrated in vacuo to yield a red oil. This oil was redissolved in benzene (100 mL) and treated again with *tert*-butyllithium (30 mL of 1.39 M). Workup as above yielded ca. 5 g of red oil. This material was chromatographed on 250 g of Woelm silica gel and eluted with 1% EtOH–CH_2Cl_2. This yielded 1.2 g of yellow solid, which was recrystallized from cyclohexane, mp 184–190 °C. In later runs we found that the crude oil from the reaction could be triturated with hot cyclohexane to yield crystallizine product: NMR ($CDCl_3$, Me_4Si) δ 1.0 (s, 9 H, *tert*-butyl), 3.7 (br d, J = 11 Hz, 1 H, PhCH-N), 4.5 (d of d, J = 10 Hz and 5 Hz, PhCHPh). Anal. ($C_{23}H_{29}NO$) C, H, N.

1-(5-Carboxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene)-4-piperidinone Ethylene Ketal (10). Anhydrous sodium carbonate (4 g) followed by 4-piperidinone ethylene ketal (6.1 g, 42.5 mmol) were added to a stirred solution of the acid chloride (10.9 g, 42.5 mmol) of carboxylic acid **9a**.[18] The mixture was stirred for 24 h (overnight) before it was diluted with ethyl acetate, and the sodium carbonate was removed by filtration. The filtrate was washed with 1.2 N hydrochloric acid, 15% aqueous sodium hydroxide, and brine, dried with $MgSO_4$, and concentrated to yield 13 g of white solid which was used without further purification. A small sample was recrystallized from cyclohexane, mp 148.5–149.5 °C. Anal. ($C_{23}H_{25}NO_3$) C, H, N.

1-(5-Methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene)-4-piperidinone Ethylene Ketal. Amide **10** (15.2 g, 41.8 mmol) in 200 mL of THF was added dropwise over a period of 40 min to a stirred suspension of lithium aluminum hydride (3.17 g, 83.6 mmol) in THF (100 mL) under reflux and an atmosphere of nitrogen. After 27 h under reflux, the mixture was cooled with an ice bath and quenched with the careful sequential addition of 3.2 mL of H_2O, 3.2 mL of 15% NaOH (aq), and 9.5 mL of H_2O. Filtration and concentration of the filtrate yielded 15 g of clear colorless oil. NMR and IR spectra indicated a considerable amount of unreacted starting material. Thus, the oil was recycled with $LiAlH_4$ (2.0 g) under reflux for 6.5 h. Workup as before yielded 14.77 g of oil which was used without further purification.

1-(5-Methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene)-4-piperidinone (11). Aqueous hydrochloric acid (50 mL of 1.2 N) was added to a stirred solution of ketal (14.45 g) in dioxane (250 mL). The mixture was heated under reflux for 6.5 h before it was concentrated on a rotary evaporator. The residue was basified with 50 mL of 15% NaOH (aq), diluted with water, and extracted three times with ether (500 mL total). The combined extracts were washed with brine, dried with $MgSO_4$, and concentrated to yield 11.2 g of clear yellow oil. This was used without further purification, since TLC (silica, 5% EtOH–CH_2Cl_2) showed (I_2) only traces of impurities. A small sample was triturated with pentane and recrystallized from isopropyl alcohol to yield a white solid, mp 78.5–80 °C. Anal. ($C_{21}H_{23}NO$) C, H, N.

Table III. Physical Characteristics of Compounds Not Specifically Cited Under the Experimental Section

compd	recrystn solvent	mp, °C	anal.
12a	sublimed	96–98.5	$C_{25}H_{33}NO$ (C, H, N)
12b[a]		239–243	$C_{25}H_{32}NOCl$ (C, H, N, Cl)
12c[a]	*i*-Pr alcohol	258–262	$C_{25}H_{33}NOCl_2$ (C, H, N, Cl)
12d[a]		275–278	$C_{25}H_{33}NOBrCl$ (C, H, N, Cl)
12e[a]		239–244	$C_{23}H_{30}NOSCl$ (C, H, N, Cl)
12f[a]	acetonitrile	246–248	$C_{23}H_{30}NO_2Cl$ (C, H, N, Cl)
14a	cyclohexane	189–190.5	$C_{25}H_{31}NO_2$ (C, H, N)
14b	CCl_4	160–163	$C_{25}H_{29}NO_2$ (C, H, N)
14d	cyclohexane	165–168	$C_{25}H_{30}NO_2Br$ (C, H, N, Br)
14e	benzene	160–162.5	$C_{23}H_{27}NO_2S$ (C, H, N, S)
14f	ethyl acetate	214.5–216	$C_{23}H_{27}NO_3$ (C, H, N)

[a] These compounds were purified as HCl salts which in most cases precipitated from solution in analytical purity after they were dried under vacuum.

1-(5-Methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene)-4-*tert*-butyl-4-piperidinol (12a). Ketone **11** (5.0 g) in benzene (150 mL) was added dropwise over 50 min to a stirred, cooled (ice bath) solution of *tert*-butyllithium (33.8 mL of 1.94 M in Et_2O) in benzene (150 mL) under nitrogen. The red solution was stirred at ambient temperature for 1 h before it was quenched with the addition of 100 mL of 10% NH_4Cl (aq). After it was stirred for 2 h, the organic layer was separated, washed with brine, dried with $MgSO_4$, and concentrated in vacuo to yield 5.6 g of clear yellow oil. This material was chromatographed on 250 g of Woelm silica gel with low-pressure liquid chromatography. Elution with 1% EtOH–CH_2Cl_2 yielded 1.5 g of pure material which could be sublimed [155 °C (0.1 mm)] to obtain an analytical sample: mp 96–98.5 °C; NMR ($CDCl_3$, Me_4Si) δ 0.9 (s, 9 H, *t*-Bu) 3.1 (d, J = 7, Hz, $-CH_2N$), 4.3 (t, J = 7 Hz, methine). Anal. ($C_{25}H_{33}NO$) C, H, N.

1-(5-Carboxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene)-4-*tert*-butyl-4-piperidinol (14a). General Procedure for 14a–f. The acid chloride (3.0 g, 11 mmol) from carboxylic acid **9a**[18] and 4-*tert*-butyl-4-piperidinol (**13**; 1.75 g, 11 mmol) were admixed in toluene (15 mL) before the addition of triethylamine (1.5 g). The resultant mixture was stirred at 75 °C under nitrogen. After 1.5 h, methylene chloride (5 mL) was added because the product precipitated and the reaction mixture set up solid. Some of these reactions were run in methylene chloride to avoid such a problem. After a total of 24 h, the mixture was concentrated on a rotary evaporator to yield a tan solid. This was redissolved in chloroform and washed with 1 N hydrochloric acid twice, saturated sodium bicarbonate solution, and brine. It was dried with $MgSO_4$ and concentrated in vacuo to yield a yellow oil. Trituration with ether yielded 3.95 g of white solid which was used without further purification. See Table III for analytical data on these intermediates, i.e., **14a–f**.

1-(5-Methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene)-4-*tert*-butyl-4-piperidinol (12a). General Procedure

for Aluminum Hydride Reductions. Concentrated sulfuric acid (0.85 mL) was added dropwise over 5 min to a cooled (ice bath) solution of lithium aluminum hydride in THF (35.3 mL of 0.9 M). The mixture was stirred at 0 °C for 1 h before the addition of the amide **14a** (3.0 g, 7.95 mmol) in THF (50 mL) over a 5-min period. The mixture was allowed to warm to room temperature and stirred for 2 days. This was out of convenience. Usual reaction times were 18–24 h. The mixture was cooled with an ice bath and quenched with the careful sequential addition of 1.2 mL of H_2O, 1.2 mL of 15% NaOH (aq), and 3.6 mL of H_2O. It was stirred for 4 h before the aluminum salts were removed by filtration and washed well with ether. Concentration of the filtrate in vacuo yielded 3.15 g of colorless glass which crystallized on sitting. See Table III for analytical data for **12a–f**.

Blockade of *d*-Amphetamine-Induced Lethality. Groups of 10 or 20 male Crl: COBS® CD®-1 (ICR) BR mice weighing 20–30 g were used in this procedure. Thirty minutes after the injection of vehicle or various doses of test drug (Table II), *d*-amphetamine (115 mg/kg ip) was given and each animal was placed in an individual observation cage. This dose of *d*-amphetamine was used to induce a rapid onset of 70–100% lethality.

Thirty minutes after *d*-amphetamine, the number of mice surviving at each test drug dose was recorded. The drug-treated groups were compared with the vehicle-treated group by means of Fisher's exact probability test, $p \leq 0.05$.

Antagonism of *d*-Amphetamine-Induced Stereotypy. Groups of ten male Crl: COBS® CD®-1 (ICR) BR mice weighing 20–30 g were brought into the testing room and allowed to acclimate for 0.5 h. Test compound or vehicle was administered by the intraperitoneal (ip) route. Immediately after this injection, each mouse was placed in a 13.1 × 13.1 × 13.1 cm Plexiglas cage with a mesh steel screen cover. Sixty minutes later, *d*-amphetamine sulfate (12.5 mg/kg ip) was given. This dose of *d*-amphetamine sulfate was shown to cause gnawing, licking, and/or sniffing in 80–100% of control animals. Each mouse was then observed for a 10-s period for continuous or intermittent gnawing, licking, and/or sniffing at 30, 60, and 90 min after *d*-amphetamine sulfate. For each behavior, the data were recorded as the difference between the drug and control groups that did not show gnawing, licking, or sniffing per groups of ten mice. ED_{50} values were calculated, if data permitted, using the Honeywell 6000 computer.

Conditioned Avoidance Response in Trained Rat. The apparatus consisted of a shuttle box divided into two compartments and enclosed in a sound attenuating chamber. The floor of the shuttle box was an electrifiable grid. Following intraperitoneal injection of the vehicle or the drug, the rat was placed into the shuttle cage and was allowed to acclimate for approximately 1 min. A 5-s conditioned stimulus, consisting of a tone and a light, preceded a 0.2-mA foot shock delivered to the grid floor of the cage. A shuttle response during the conditioned stimulus period terminated the conditioned stimulus, prevented the onset of the shock, and was scored as an avoidance response. A shuttle response during the shock period terminated the conditioned stimulus and the shock was scored as an escape response. If no escape response was made, an escape failure was scored. Each conditioned stimulus presentation was separated by a 15-s interval, and a response during this time resulted in the onset of the shock and the conditioned stimulus until the rat returned to the other side. Each rat was presented with 100 trials (i.e., 100 conditioned stimulus presentations) which took approximately 30 min. Each rat was trained to an average criterion of ≥85 avoidance responses per 100 trial session when administered vehicle control solution. Eight CD®F strain male albino rats per dose were tested, and each rat's performance under the drug treatment was compared to his previous performance under vehicle control treatment. Comparisons were made by means of a paired Student's *t* test ($p \leq 0.05$, two tailed). Two or more doses of a compound were evaluated. All drugs were evaluated at 30-min postinjection.

Receptor Binding Studies. Calf caudate nuclei were dissected from freshly obtained brains and stored frozen at −76 °C. As needed, caudate tissue was homogenized and prepared following procedures outlined by Creese and co-workers.[6]

Receptor binding studies were performed as reported in the literature[6,8,10,11] with slight modifications. A typical sample contained 2 mL of caudate membrane homogenate (10 mg of original tissue/mL) in a final ligand concentration of either 2.5 nM [^{3}H]dopamine, 1.6 nM [^{3}H]haloperidol, or 0.15 nM spiroperidol. Test compounds were added as 20-μL aliquots from stock solutions prepared in absolute ethanol or 0.1% ascorbic acid. Samples were incubated in triplicate at 37 °C for 10 min when [^{3}H]dopamine or [^{3}H]haloperidol was used and for 20 min when [^{3}H]spiroperidol was present.

Immediately following all incubations, proteins were recovered on Whatman GF/B glass-fiber filters under reduced pressure. Trapped membranes were solubilized off the filters using 1 mL of NCS tissue solubilizer (Amersham/Searle Corp.) at 50 °C for 1 h. Then the pH was adjusted by adding 0.1 mL of glacial acetic acid, 10 mL of PCS (Amersham/Searle Corp.) was added, and the samples were analyzed for membrane-bound radioactivity using a Mark II liquid scintillation counter (Searle Analytical, Inc.).

Nonspecific binding was measured in the presence of 10^{-5} M (+)-butaclamol for the [^{3}H]dopamine and [^{3}H]spiroperidol studies and 10^{-4} M nonradiolabeled dopamine for the [^{3}H]haloperidol studies. IC_{50} values were determined from log probit using four to six concentrations of each compound.

Acknowledgment. Appreciation is extended to Mr. C. Woo for chemical assistance, Drs. C. R. Ellefson and F. M. Hershenson for helpful discussions, Mr. E. Zielinski for microanalytical results, Mr. C. Rothman for chromatographic separations, and Ms. J. Vassilatos and Ms. P. Evanco for preparation of the manuscript.

References and Notes

(1) (a) This paper was presented in part at the 16th National Medicinal Chemistry Symposium, Kalamazoo, Mich., June 19–22, 1978. (b) Address correspondence to this author at McNeil Laboratories, Fort Washington, Pa.

(2) (a) R. Matz, W. Rick, D. Oh, H. Thompson, and S. Gershon, *Curr. Ther. Res.*, **16**, 687 (1974); (b) G. M. Simpson, J. H. Lee, and R. K. Shivastava, *Psychopharmacologia*, **56**, 75 (1978); (c) N. Nair, V. Zicherman, and G. Schwartz, *Can. Psychiatr. Assoc. J.*, **22**, 285 (1977).

(3) J. Idänpään-Heikkilä, E. Alhava, M. Olkinuora, and I. Palva, *Eur. J. Clin. Pharmacol.*, 11, 193 (1977).

(4) M. L. Clark, A. Paredes, J. Costiloe, and F. Wood, *J. Clin. Pharm.*, 17, 529 (1977).

(5) S. H. Snyder, I. Creese, and D. R. Burt, *Psychopharmacol. Commun.*, 1, 663 (1975).

(6) I. Creese, D. R. Burt, and S. H. Snyder, *Life Sci.*, 17, 993 (1975).

(7) P. Seeman, M. Chou-Wong, J. Tedesco, and K. Wong, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 4326 (1975).

(8) D. R. Burt, S. J. Enna, I. Creese, and S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 4665 (1975).

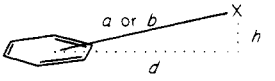
(9) I. Creese, D. R. Burt, and S. H. Snyder, *Science*, **192**, 481 (1976).

(10) D. R. Burt, I. Creese, and S. H. Snyder, *Mol. Pharmacol.*, **12**, 800 (1976).

(11) J. Leysen, W. Gommersen, and P. Laudron, *Biochem. Pharmacol.*, **27**, 307 (1978).

(12) P. Laudron, P. Janssen, and J. Leysen, *Biochem. Pharmacol.*, **27**, 317, 323 (1978).

(13) The rigid nature of these molecules validates the use of Dreiding models to measure and compare intramolecular distances. The distances as shown (*a* and *b*) are quantitatively best characterized by division into a height (above the plane of the phenyl ring) and distance component, i.e.,



In the case of clozapine, the distances are 5.0 (*d*) and 0.8 Å (*h*) for *a* and 5.7 (*d*) and 1.5 Å (*h*) for *b*. Butaclamol has two possible twist conformations of the ethylene bridge of the central seven-membered ring. Since there are no discernable interactions which would favor one over the other, the one which most closely resembles clozapine was chosen for measurement purposes. This indicated 4.8 (*d*)

and 0.9 Å (*h*) for *a* and 6.1 (*d*) and 0.15 Å (*h*) for *b*.
(14) R. Unger, S. Sommer, E. Schorscher, and H. Muller-Calgan, U.S. Patent 3 393 198 (1968).
(15) N. Malatestinic and A. Ziering, U.S. Patent 3 679 666 (1972).
(16) F. T. Bruderlein, L. G. Humber, and K. Pelz, *Can. J. Chem.*, **52**, 2119 (1974).
(17) P. H. Bird, F. T. Bruderlein, and L. G. Humber, *Can. J. Chem.*, **54**, 2715 (1976).
(18) The source of the carboxylic acids **9a–f** is as follows: (a) **9a**; M. Davis, S. Winthrop, J. Stewart, F. Sunahara, and F. Herr, *J. Med. Chem.*, **6**, 251 (1963). (b) **9b**, **9c**[19] and **9d**[19] were synthesized by the general method of M. Davis, S. Winthrop, R. Thomas, F. Herr, M.-P. Charest, and R. Gaudry, *J. Med. Chem.*, **7**, 88 (1964), in which the tricyclic 5-ol was converted to the chloride, then to the nitrile, and subsequently hydrolyzed. (c) **9e**; R. Burtner and J. Cusic, *J. Am. Chem. Soc.*, **65**, 1582 (1943).
(19) The dibenzosuberone derivatives which were reduced to the 5-ol compounds as precursors for **9c** and **9d** are described in E. Englehardt, H. Zell, W. Saari, M. Christy, C. Colton, C. Stone, J. Stavorski, H. Wenger, and C. Ludden, *J. Med. Chem.*, **8**, 928 (1965).
(20) References to each of the tests used in pharmacological evaluation of these compounds can be found in "*Industrial Pharmacology*", Volume 1, Neuroleptics, S. Fielding and H. Lal, Ed., Futura Publishing Co., Mount Kisko, N.Y., 1974.

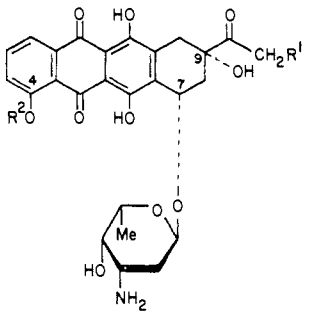
Synthesis and Antitumor Activity of Sugar-Ring Hydroxyl Analogues of Daunorubicin[1]

Ernst-F. Fuchs, Derek Horton,* Wolfgang Weckerle, and Eva Winter-Mihaly

Department of Chemistry, The Ohio State University, Columbus, Ohio 43210. *Received August 4, 1978*

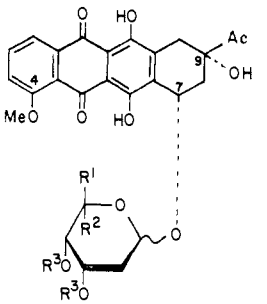
Daunorubicin analogues in which the natural amino sugar, daunosamine, is replaced by neutral 2,6-dideoxyhexopyranosyl residues have been prepared in high yields. Glycosidation of 3,4-di-*O*-acetyl-2,6-dideoxy-α-L-*lyxo*-hexopyranosyl chloride (**13**) with daunomycinone under Koenigs–Knorr conditions yielded exclusively the protected α-anomeric product **4**, which was converted into the free glycoside **5**. In contrast, the 1-chloro-D-*ribo* isomer **19**, bearing *p*-nitrobenzoyl groups for hydroxyl-group protection, furnished a 5:3 mixture of the α (**6**) and β (**7**) glycosides. Separation and individual deprotection afforded the target compounds **8** (from **6**) and **9** (from **7**). Whereas all of the D-*ribo* analogues (**6–9**) are inactive as antitumor agents in vivo against P388 lymphocytic leukemia in mice, the protected L-*lyxo* glycoside **4** (T/C 186) and also the free glycoside **5** (T/C 183) are highly effective in this test system; **5** is also active (T/C 146) in vivo against murine B16 melanocarcinoma.

The anthracycline antibiotics[2,3] daunorubicin (**1**; NSC-82151) and adriamycin (doxorubicin, **2**; NSC-123127)



1, $R^1 = H$; $R^2 = Me$
2, $R^1 = OH$; $R^2 = Me$
3, $R^1 = H$; $R^2 = H$

are potent and clinically useful antitumor agents. Similarly, the closely related carminomycin (**3**; NSC-180024) is considered a highly promising anticancer drug under clinical trial. Their broader utilization in chemotherapy is hampered,[4] however, by their scarcity and by certain undesirable side effects common to many antitumor drugs (such as bone-marrow damage, stomatitis, alopecia, and mutagenic behavior) but, in particular, a cumulative, dose-limiting cardiotoxicity (congestive heart failure). The scarcity factor has led to continuing efforts toward the total synthesis of **1–3** as a more economical alternative to the fermentation route, and the undesirable side effects have stimulated increasing interest in the preparation of analogues (derivatives of the parent antibiotics and semisynthetic and totally synthetic analogues) that may display more favorable therapeutic characteristics than the parent drugs. As part of our program aimed toward the development of new semisynthetic analogues, we now report the synthesis of 7-*O*-(2,6-dideoxy-α-L-*lyxo*-hexopyranosyl)daunomycinone ("3′-hydroxydaunorubicin", **5**;



	Anomer	R^1	R^2	R^3
4	α	H	Me	Ac
5	α	H	Me	H
6	α	Me	H	$O_2NC_6H_4CO$
7	β	Me	H	$O_2NC_6H_4CO$
8	α	Me	H	H
9	β	Me	H	H

NSC-284682) in which the natural amino sugar, daunosamine (3-amino-2,3,6-trideoxy-L-*lyxo*-hexose), in **1** has been replaced by the corresponding 3-hydroxyl analogue (2-deoxy-L-fucose, **10**). Biological evaluation of **5** was expected to shed some light on the role of the amino group at C-3 of the sugar moiety. Structural features of the sugar component are considered to exert a decisive influence on the pharmacological properties of members of this class of antitumor drugs. In addition, we present here the synthesis and results of an initial biological evaluation of the anomeric glycosides **8** (NSC-294987) and **9** (NSC-297279) in which 2,6-dideoxy-D-*ribo*-hexose (digitoxose, **14**)

0022-2623/79/1822-0406$01.00/0