Cycloheximide Analogues as Potential Anticonvulsants

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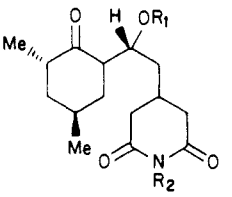
A series of 22 cycloheximide analogues in which the substituents on the cycloheximide ring and imide nitrogen were varied, the glutarimide ring was changed to a succinimide ring, and the ring and/or side-chain oxygens were present as ketone and/or alcohol groups were prepared and sent to the Anticonvulsant Drug Development program of the National Institute of Neurological and Communicative Disorders and Strokes for evaluation as anticonvulsants. Three compounds, namely cycloheximide (**1a**), 2-methyl dione **2c**, and dihydrocycloheximide (**4a**), were further evaluated in Phase II testing for quantification of maximum activity with the latter eventually progressing to Phase IV and Phase VI screens.

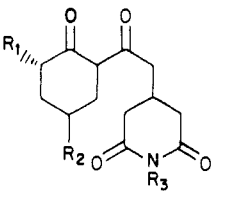
Cycloheximide (**1a**) is a relatively simple antibiotic when compared to many, but yet it has a wide spectrum of biological activities and effects.[1] Much of the biologically related research on cycloheximide has focused upon its inhibition of DNA and RNA biosynthesis and its stimulation of interferon formation, but the biological properties of cycloheximide and some analogues have even extended to the prevention of proliferative skin diseases[2] and to antiviral activity.[3]

Previously, we[4] had tried to utilize the salient features of cycloheximide which were considered to be essential to its biological action[5] by incorporating them into heterocyclic rings with the intention of preparing potential antitumor agents. These target compounds were inactive, however, in the L1210 and Walker 256 cancer test systems. Later, some of the diketone precursors **2** and **3**, prepared during the course of this prior study along with some dialcohol derivatives **4**, were submitted to the Anticonvulsant Drug Development (ADD) Program of the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS). A few derivatives had favorable anticonvulsant activity which prompted the preparation of several more analogues to secure preliminary information about this class of compounds. The only other indication of this type of effect was given by Ogata,[6] who examined the effect cycloheximide would have on experimental epilepsy induced in rabbits by daily amygdaloid stimulation. Also, glutarimides substituted on the nitrogen and/or C-4 by alkyl groups have led to compounds with some anticonvulsant and hypnotic action.[7-9]

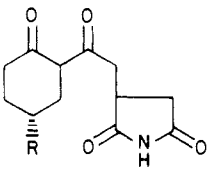
Chemistry. The 1,3-diketones **2** and **3** were synthesized from the enamine of the appropriately substituted ketone and the acid chloride of 2,6-dioxo-4-piperidineacetic and/or 2,5-dioxo-3-pyrrolidineacetic acid essentially as described,[10]



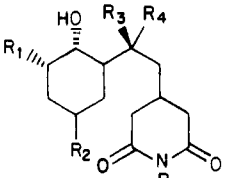
1a, $R_1 = R_2 = H$
b, $R_1 = Ac$; $R_2 = Me$



2a, $R_1 = R_2 = R_3 = H$
b, $R_1 = Me$; $R_2 = ax\ Me$; $R_3 = H$
c, $R_1 = Me$; $R_2 = R_3 = H$
d, $R_1 = R_3 = H$; $R_2 = eq\ Me$
e, $R_1 = R_2 = H$; $R_3 = Me$
f, $R_1 = R_3 = Me$; $R_2 = eq\ Me$
g, $R_1 = R_2 = H$; $R_3 = Me$
h, $R_1 = H$; $R_2 = eq\ Me$; $R_3 = Me$



3a, R = H
b, R = Me



4a, $R_1 = Me$; $R_2 = ax\ Me$; $R_3 = R_5 = H$; $R_4 = OH$
b, $R_1 = Me$; $R_2 = R_3 = R_5 = H$; $R_4 = OH$
c, $R_1 = R_2 = R_5 = H$; R_3 or $R_4 = H$ or OH
d, $R_1 = R_5 = H$; $R_2 = eq\ Me$; R_3 or $R_4 = H$ or OH
e, $R_1 = R_2 = H$; R_3 or $R_4 = H$ or OH; $R_5 = CH_2Ph$
f, $R_1 = R_2 = R_4 = H$; $R_3 = OH$; $R_5 = CH_2Ph$
g, $R_1 = R_2 = R_3 = H$; $R_4 = OH$; $R_5 = CH_2Ph$
h, $R_1 = R_5 = Me$; $R_2 = ax\ Me$; $R_3 = H$; $R_4 = OH$
i, $R_1 = R_2 = H$; R_3 or $R_4 = H$ or OH; $R_5 = Me$
j, $R_1 = R_5 = Me$; $R_2 = R_3 = H$; $R_4 = OH$
k, $R_1 = H$; $R_2 = eq\ Me$; R_3 or $R_4 = H$ or OH; $R_5 = Me$
l, $R_1 = Me$; $R_2 = ax\ Me$; $R_3 = H$; $R_4 = OH$; $R_5 = CH_2Ph$

except the modifications introduced by Johnson et al.[11] in the total synthesis of cycloheximide were used to obtain better yields. Stereochemical considerations of the cyclohexane ring when C-2 substituents are present follow from this latter work.[11] The pyrazole **5** was prepared by us before.[4]

The 1,3-diols **4** were secured from the corresponding ketones **2** by catalytic reduction over Pt in glacial HOAc as described.[11] Cycloheximide, though, was reduced in the same manner to provide its diol **4a**. Formation of the appropriate diols was verified through their IR absorption at 3350–3500 cm^{-1} and ^1H NMR signals at δ 3.95–4.18 and 3.92–3.98 for the protons on the two alcohol-bearing carbons. The stereochemistry of the 2-methyl diol **4b** and cycloheximide diol **4a** follows from the arguments of Johnson et al.[11] in their total synthesis of cycloheximide.

Without the methyl moiety on C-2 of the cyclohexanone ring to influence stereochemical orientations during acy-

(1) Reviews concerning biological action may be found in the following: Litinshaya, T. K. *Mol. Biol. (Kiev)* **1978**, *20*, 74; *Chem. Abstr.* **1979**, *90*, 975. Sisler, H. D.; Siegel, M. R. *Antibiotics* **1967**, *1*, 283. For a complete review of cycloheximide chemistry, see: Johnson, F. *Fortsch. Chem. Org. Naturst.* **1971**, *29*, 140. More recently, a review on cycloheximide isolation, biosynthesis, and properties has appeared by Jost et al.: Jost, J. L.; Kominek, L. A.; Hyatt, G. S.; Wang, H. Y. *Drugs Pharm. Sci.* **1984**, *22*, 531.
(2) Montes, F. L. German Patent 2944587, 1980; *Chem. Abstr.* **1978**, *88*, 6597.
(3) (a) Makaiyama, T.; Wagatsuma, K. *Japanese* Patent 7783570, 1972; *Chem. Abstr.* **1978**, *88*, 22635. (b) *Ibid.* 7783571, 1972; *Chem. Abstr.* **1978**, *88*, 22638.
(4) Piatak, D. M.; Yen, C. C.; Kennedy, R. V., Jr. *J. Med. Chem.* **1970**, *13*, 770.
(5) (a) Grollman, A. P. *Science* **1967**, *157*, 84. (b) Siegel, M. R.; Sisler, H. D.; Johnson, F. *Biochem. Pharmacol.* **1966**, *15*, 1213.
(6) Ogata, N. *Epilepsia* (N.Y.) **1977**, *181*, 101; *Chem. Abstr.* **1980**, *92*, 157759.
(7) Laycock, G. M.; Shulman, A. *Nature (London)* **1963**, *200*, 849.
(8) Shulman, A. *Proc. R. Aust. Chem. Inst.* **1964**, *31*, 41.
(9) Cameron, D. W.; Down, J. G.; Kissane, P. S.; Laycock, G. M.; Shulman, A. *Aust. J. Chem.* **1977**, *30*, 1157.
(10) Struck, R. F.; Schaeffer, H. J.; Krauth, C. A.; Kemp, R. J.; Shealy, Y. F.; Montgomery, S. A. *J. Med. Chem.* **1964**, *7*, 646.
(11) Johnson, F.; Starkovsky, N. A.; Carlson, A. A. *J. Am. Chem. Soc.* **1965**, *87*, 4612. Johnson, F.; Starkovsky, N. A.; Paton, A. C.; Carlson, A. A. *J. Am. Chem. Soc.* **1966**, *88*, 149.

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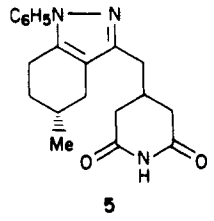
lation of the enamine and later catalytic reduction of the dione, the conformation of diols **4c** and **4d** cannot be assigned a priori. Therefore, the reduction products of diketone **2a**, after N-benzylation of the unrecrystallized imide **4c**, were examined to establish how many and which isomers resulted. With the crude benzylated product it would be possible to effect an easier chromatographic separation and to obtain an overall view of all the isomer forms. The results would also be applicable to the 4-methyl diol **4d**, which was assumed to have the methyl group oriented equatorially throughout the synthesis as a consequence of 1,3-diaxial steric interactions.

Preparative TLC of the N-benzylation reaction product **4e** gave five compounds, the most polar of which was noncrystalline but which was identified as lactone amide **6a** on the basis of spectral data akin to that of the characterized amide **6b** (see below). The other four compounds consisted of two pairs of isomers according to similarities in IR and ^{1}H NMR spectra. The less polar pair **7a** and **7b** had single OH stretching bands in an IR at 3380 and 3420 cm^{-1}, respectively, three distinct carbonyl bands at 1720, 1700, and 1660 cm^{-1}, and ^{1}H NMR signals at δ 3.71 and 4.18, respectively, for CHO protons. The more polar pair exhibited two OH bands at 3450 and 3370 cm^{-1} for **4f** and at 3500 and 3400 cm^{-1} for **4g** and only two carbonyl absorptions at 1728 and 1673 cm^{-1}. Two separate NMR signals for protons on hydroxyl group bearing carbons were also noted at δ 4.23 and 3.65 for **4f** and at δ 4.1 and 3.9 for **4g**. The number of hydroxyl groups in each pair was verified through formation of acetate derivatives and analysis of ^{1}H NMR spectra taken in $CDCl_3$ and C_6D_6. The less polar pair also had ^{13}C NMR signals for a ketone and an alcohol carbon while the more polar pair had two signals for alcohol carbons, confirming hydroxy ketone structures for the more mobile pair and 1,3-diol structures for the latter. The hydroxy ketone system was judged to be the pseudocycloheximide type because it would more likely survive the strongly basic conditions and workup of the N-benzylation which are known to cause cycloheximide to undergo a retroaldol reaction or a dehydration.



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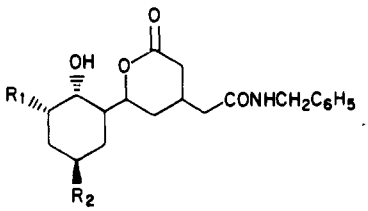
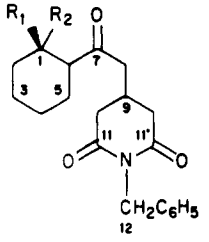


6a, $R_1 = R_2 = H$
b, $R_1 = R_2 = Me$



7a, R_1 = OH; R_2 = H
b, R_1 = H; R_2 = OH

Analysis of the stereochemistry of **7a** and **7b** was predicated upon the assumption that such a bulky side chain would favor the less hindered equatorial orientation, particularly since enolization of the ketone could easily allow such a conformational change. From the broad signal at δ 3.71 for **7a** and the sharp one at δ 4.18 for **7b** together with observations by Johnson et al.[11] for cycloheximide reduction products, an axial C-1 proton or equatorial OH was assigned to **7a** while an equatorial C-1 proton or axial OH was attributed to **7b**. Confirmation was secured from ^{13}C NMR data whereby the alcohol-bearing carbon for **7a** was at a lower field in accordance with general observations[12g,13] on cyclohexane substituents.

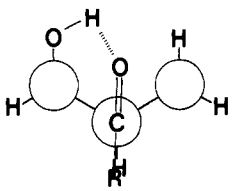
Table I. Data for New Methylated Diketone, Diol, and Methylated Diol Analogues

compd	mp, °C	yield,[a] %	formula[b]
2f	122.5–123.5	44.5[c]	$C_{16}H_{25}NO_4$
2g	94–94.5	26[d]	$C_{15}H_{23}NO_4$
2h	159–161	38.3[c]	$C_{15}H_{23}NO_4$
4b	140–142	50[e]	$C_{14}H_{23}NO_4$
4d	162–163	65[e]	$C_{14}H_{23}NO_4$
4j	135.5–137.5	33[f]	$C_{15}H_{25}NO_4$
4k	117.5–119	35[g]	$C_{15}H_{25}NO_4$

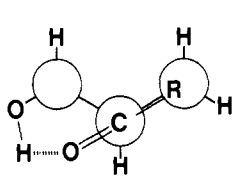
[a] Based upon recrystallized yields. [b] All compounds gave satisfactory analyses for C, H, N (±0.4%) and had approproate IR and ^{1}H NMR data. [c] From MeOH–$CHCl_3$. [d] From MeOH. [e] From EtOAc. [f] From CH_2Cl_2–hexane. [g] From MeOH–EtOAc.

For 1,3-diols **4f** and **4g** the side chain was again considered as favoring the equatorial conformation particularly since hydroxy ketones appear to be intermediates. ^{1}H NMR spectra of both featured a broad upfield signal (δ 3.65 and 3.9) and a sharper downfield signal (δ 4.23 and 4.1) for the side chain and ring CHO protons, respectively. From the data the ring OH was considered to be axial since ring equatorial protons of this type generally appear more downfield than axial protons.[14] The signals were also not as broad as the one for the axial proton in **7a**. The side-chain OH configurations were secured by comparison of the CHO proton chemical shifts in $CDCl_3$ and C_6D_6 with those of catalytically reduced cycloheximide and were assigned as axial-erythro and axial-threo, respectively, as shown in **4f** and **4g**. ^{13}C NMR chemical shift data supported the assignments. Therefore, the crystallized product used in the bioassay is a mixture of **4f** and **4g**.

To rationalize the formation of these compounds the results were viewed in light of the stereochemical arguments presented in the synthesis of cycloheximide. In this instance reduction of the ring ketone would be expected to occur from either face of the ring in the absence of the methyl moiety α to the ketone which controlled the orientation of the reduction in cycloheximide. The bulky side chain can then orient equatorially to give hydroxy ketones **7a** and **7b** either through enolization of the ketone or by an interconversion of the ring from one chair form to another. Reduction of the side-chain ketone would then take place to yield diols **4f** and **4g**. This latter reduction apparently favors isomer **7b**, which allows for an easier reduction in the more accessible hydrogen bonded conformer A than does isomer **7a**, which gives conformer B with its adjacent axial hydrogens.



A



B

To obtain *N*-methyl derivatives **2e-h** and **4h-k** and thus test the consequences of modifying the imide nitrogen

(12) Jeffs, P.; McWilliams, D. *J. Am. Chem. Soc.* **1981**, *103*, 6185.
(13) Abraham, R. J.; Loftus, P. "Proton and Carbon-13 NMR Spectroscopy"; Heydon: London, 1980; p 29.
(14) Eliel, E. L.; Gianni, M. H.; William, T. H.; Stothers, J. B. *Tetrahedron Lett.* **1962**, 741.

Table II. Phase I Anticonvulsant Testing Data[a]

compd	data[b]	MES[c] 0.5 h	MES[c] 4 h	scMet[d] 0.5 h	scMet[d] 4 h	tox[e]	scMet repeat[f] 0.5 h	scMet repeat[f] 4 h	tox[e]
1a	commercial	+	-	+++	++	1	+++	++	0
1b	15	-	-	-	-				
2a	10	-	-	+	-	0	+		
2b	4, 15	-	-	-	-				
2c	10	-	-	++	-	0	++[g]		0
2d	10	-	-	-	-				
2e		-	-	-	-				
2f		-	+	+	-	1	+		1
2g		+	-	+	-	1	+		3
2h		-	-	-	-				
3a	10	-	-	+	-	0	+		0
3b	10	+	-	+	-	2	+		1
4a	11, 15	-	-	+++	+	1	+++	+[h]	0
4c		-	-	+++	-	0	+		0
4d		-	-	++++	+	0	++++[i]	-	0
4h	15	+	-	-	-	0			
4i		-	-	-	-				
4j		+	-	-	-	0			
4k		+	-	-	-	4			
4l		-	-	-	-				
5	4	-	-	-	++++	0	-		
6b		-	-	-	-				

[a] ++++, +++, ++, and + denotes activity at 30, 100, 300, or 600 mg/kg, respectively, - denotes inactivity or no toxicity up to 600 mg/kg. [b] Reference number for compound if prepared previously. [c] Activity in the maximal electroshock seizure test at 0.5 and 4 h after administration. [d] Activity in the subcutaneous pentylenetetrazol seizure test at 0.5 and 4 h after administration. [e] Number of animals out of four exhibiting toxicity in the rotorod test. [f] Lowest dose at which at least half of the four animals used were protected. [g] Rescreened at 100, 250, 450, and 600 mg/kg with two, three, four, and four animals protected, respectively, and no toxicity indicated. [h] Only three animals used. [i] Rescreened at 10, 20, 30, 100, and 300 mg/kg with zero, zero, one, one, and one animals protected, respectively.

upon the biological effects of these compounds, various diketone and diol analogues were treated with CH_2N_2 as done previously with cycloheximide.[15] Although the yields were poor, the procedure gave no side products and the desired compounds were readily isolated (see Table I).

The ease of N-alkylation with base and an alkyl halide was also tested by reacting dihydrocycloheximide with KO-*t*-Bu and $C_6H_5CH_2Cl$. The expected product **4l** and amide lactone **6b** resulting from glutarimide ring fission were isolated. Identity of the *N*-benzyl glutarimide **4l** was established by loss of the NH IR bands at 3100 and 3200 cm^{-1}, appearance of characteristic benzene peaks at 1490 and 1580 cm^{-1}, and retention of the 1700 and 1665 cm^{-1} peaks for the glutarimide ring. A ^1H NMR spectrum had a five-proton singlet at δ 7.20 and a singlet at δ 4.95 for the benzylic protons.

The amide lactone **6b**, which ensues from glutarimide ring fission in aqueous acidic or basic reaction media or workup,[15] was characterized from IR bands at 1720 cm^{-1} for the lactone carbonyl and at 3340 and 1555 cm^{-1} for the secondary amide and from ^1H NMR signals at δ 7.28 for the 5 H's of the benzene ring and at δ 4.43 (doublet) for the benzylic protons. The latter signal was noted to be upfield from the singlet for the benzylic protons in glutarimide 4l, a consequence of losing the deshielding effect of the second carbonyl group.

Biological Results. Of the 22 compounds sent to the Antiepileptic Drug Development Program, 10 exhibited enough activity in initial Phase I testing for repeat assays to be performed (see Table II). From these 10 only three were selected for Phase II testing in order to quantify the effect against seizures from subcutaneous pentylenetetrazol (scMet) and/or maximal electroshock (MES) administration and neurotoxicity. Results with cycloheximide (**1a**) in the scMet screen showed no selectivity since an ED_{50} of 5.66 mg/kg (peak effect time of 1 h) was accompanied by a 95% confidence interval (CI) of 0.038–1759.3 mg/kg. Also, it was toxic at levels of 600 mg/kg and higher. For 2-methyl dione **2c** a scMet ED_{50} of 238.5 (CI 157.3–367.2) mg/kg at a peak effect time of 1 h indicated fair selectivity. No toxicity was found up to 1000 mg/kg. Neither of these compounds was selected for further study in the ADD program.

The third compound, dihydrocycloheximide (**4a**), had a MES ED_{50} of 510.0 (CI 436.2–579.3) mg/kg and a neurotoxicity TD_{50} of 746.6 (CI 672.9–842.9) mg/kg at a peak effect time of 0.5 h. The scMet test results indicated little selectivity with an ED_{50} of 50.6 (CI 13.9–93.7) mg/kg at a peak effect time of 1 h. Dihydrocycloheximide was further evaluated in Phase IV, po administration in mice, and Phase VI, oral administration in rats. However, the data showed **4a** had incomplete effectiveness since it afforded no more than 40% protection in the Phase IV scMet screen, no protection in the Phase VI scMet screen, and no more than 50% protection in both MES screens with dose levels as high as 1000 mg/kg. No further testing was scheduled in the ADD program.

The lack of specificity with these compounds indicates that this class of compounds has little anticonvulsant potential at present. It is possible the rather large substituent on the glutarimide ring limits potency since glutarimides with the relatively smaller methyl, ethyl, and/or butyl groups at the β-position (e.g., bemegride),[7-9] ethyl and substituted phenyl groups at the α-position (glutethimide and others).[16] and an α-amino group and various *N*-phenyl moieties[17] are quite potent CNS agents. Several more models will be needed to clarify this point as well as provide new directions for cycloheximide modifications.

If the data in Table II is compared, even though it is normally used only to identify compounds with anticonvulsant activity, a methyl group at C-2 of the cyclohexane ring of these compounds appears to favor activity while

(15) Kornfeld, E. C.; Jones, R. G.; Parke, T. V. *J. Am. Chem. Soc.* 1949, *71*, 150.

(16) Aboul-Enein, H. Y.; Schauberger, C. W.; Hansen, A. R.; Fischer, L. J. *J. Med. Chem.* **1975**, *18*, 736.

(17) Witiak, D. T.; Cook, W. L.; Gupta, T. K.; Gerald, M. C. *J. Med. Chem.* **1976**, *19*, 1419.

a methyl or a benzyl group on the nitrogen decreases or negates potency. Unfortunately, a key compound, the 2-methyl diol **4b**, could not be assayed because of its insolubility in the solutions employed.

Experimental Section

Melting points were determined on a Fisher-Johns block and are uncorrected. Satisfactory IR (Sargent-Welch 3-200 spectrophotometer, KBr) and NMR (IBM-NR80 spectrometer, Me_4Si as internal standard, $CDCl_3$ unless otherwise noted) spectra were obtained for all compounds, and spectral data for the compounds in Table I correlated closely to that of products from typical experiments described below. TLC sheets of silica gel 60 F_{254} from EM Reagents were used for qualitative information, and preparative TLC plates (0.5 mm) were prepared from their silica gel PF_{254}.

Reduction of Dehydrocycloheximides. In a typical experiment dehydrocycloheximide **2a** (5.0 g) and PtO_2 (0.5 g) in glacial HOAc (80 mL) were stirred under H_2 initially at 44 psi until H_2 absorption ceased (48–72 h). The catalyst was collected on Celite, the HOAc was removed in vacuo, and the residue was recrystallized from EtOAc to yield 3.25 g (65%) of diol **4c**: mp 141–142 °C; IR 3400, 3200, 3090, 1720, 1695 cm^{-1}; NMR δ 8.08 (br, 1 H), 4.2 (m, 1 H), 3.3 (d, 1 H, J = 8 Hz), 3.5 (m, 1 H). Anal. ($C_{13}H_{21}NO_4$) C, H, N. Other new diols are listed in Table I.

N-Methylation of Dehydrocycloheximides. An ether solution of CH_2N_2 (5 equiv) from Diazald was added to dehydrocycloheximide (**2a**; 2.50 g) in MeOH (50 mL), and the solution was stored overnight at room temperature. The excess CH_2N_2 and solvent were evaporated, and the resultant clear oil was separated on preparative TLC (2% MeOH/$CHCl_3$). Recrystallization of the recovered product **2e** from MeOH/$CHCl_3$ gave 0.67 g (25.4%): mp 148.5–150 °C; IR 1710, 1695, 1600 cm^{-1}; NMR δ 3.15 (s, 3 H). Anal. ($C_{14}H_{21}NO_4$) C, H, N. Table I lists other new compounds.

N-Methylation of Diols. Five equivalents of CH_2N_2 was added to 1.3 g of diol **4c** in 25 mL of MeOH, and the solution was stored overnight. After evaporation of the solvents, the residue was taken up in ether and washed with cold 10% NaOH to remove unreacted imide, and the organic layer was dried ($NaSO_4$). Recrystallization of the resultant **4i** from EtOAc–hexane gave 0.41 g (30%): mp 122–124 °C; IR 3420, 1715, 1690, 1655 cm^{-1}; NMR δ 4.2 (br, 1 H), 3.97 (d, 1 H, J = 7.8 Hz), 3.15 (s, 3 H). Anal. ($C_{14}H_{23}NO_4$) C, H, N. Data for **4j** and **4k** are listed in Table I.

N-Benzylation of Dihydrocycloheximide (4a). Dihydrocycloheximide (**4a**; 2.5 g, 8.8 mmol) was added to a stirred solution of KO-*t*-Bu (1.1 g, 9.5 mmol) in dry DMF (70 mL) under N_2. After 0.5 h $C_6H_5CH_2Cl$ (1.03 g) in DMF (20 mL) was added dropwise, and then the mixture was heated at 100 °C for 24 h. The cooled reaction mixture was diluted with 10% HCl (100 mL), the product was extracted into EtOAc and then washed with cold 10% NaOH and water, and the organic layer was dried (Na_2SO_4). Evaporation of the solvent and other volatile organics under low pressure gave a residue which was separated on preparative TLC by 5% MeOH/$CHCl_3$ into two fractions. The less polar fraction (0.71 g) was recrystallized from $CHCl_3$ to afford *N*-benzyl diol **4l** (0.42 g, 12.7%): mp 137.5–138 °C; IR 3560, 3480, 1710, 1665, 1580, 1490 cm^{-1}; NMR δ 7.28 (br s, 5 H), 4.95 (s, 2 H), 3.99 (d, 1 H), 3.8 (br s, 1 H), 0.97 (d, 3 H, J = 6.7 Hz), 0.90 (d, 3 H, J = 5 Hz). Anal. ($C_{22}H_{31}NO_4$) C, H. N.

The more polar material (0.97 g) was recrystallized from $CHCl_3$ to yield amide lactone **6b** (0.63 g, 19%): mp 151–154 °C; IR 3470, 3340, 1720, 1700, 1655, 1600, 1555, 1495 cm^{-1}; NMR δ 7.3 (s, 5 H), 6.0 (br, 1 H), 4.43 (d, 2 H, J = 5.6 Hz), 4.3 (br, 1 H), 3.73 (br, 1 H), 0.98 (d, 3 H, J = 6.8 Hz), 0.91 (d, 3 H, J = 4 Hz). Anal. ($C_{22}H_{31}NO_4$) C, H, N.

N-Benzylation of Diol 4c. A sample of unrecrystallized **4c** (2.5 g) was benzylated as above with 1 equiv of $C_6H_5CH_2Cl$. The crude product was separated first into three fractions by preparative TLC (5% MeOH/$CHCl_3$). One of the fractions was identified as amide lactone **6a**, although it did not crystallize, by IR (3450, 3300, 1720, 1650, 1540 cm^{-1}) and NMR (δ 7.5 (s), 4.36 (d, 2 H, J = 5.5 Hz)).

The other two fractions were rechromatographed on preparative TLC with 2.5% and 10% MeOH/$CHCl_3$ to give two sets of isomers which were separated into four compounds on preparative TLC with 4% MeOH/$CHCl_3$ (three dips). All four were found to be single spots on analytical TLC (10% MeOH/$CHCl_3$) with R_f values of 0.39 and 0.45 for one pair and 0.28 and 0.33 for the second. Each was then recrystallized from EtOAc. Data for each follows.

Table III. ^{13}C NMR Data for N-Benzylation Products of **2a**

	chemical shift, δ				
carbon[a]	**7a**	**7b**	**4f**	**4g**	**4a**[b]
1	71.17	66.48	71.72	72.57	74.85
2	34.48	32.17	33.14	33.90	40.67
3	24.55	22.89	24.52	19.52	33.30
4	24.22	19.58	20.09	18.48	27.02
5	27.65	24.62	25.13	25.40	24.22
6	58.43	53.90	38.42	38.33	31.02
7	211.51	212.30	67.59	71.96	72.39
8	42.64	42.73	42.67	42.61	39.34
9	24.86	25.01	26.35	26.19	27.32
10	38.27	38.33	39.85	39.34	37.06
10′	38.27	38.33	39.70	39.46	38.15
11	171.60	171.42	172.18	172.24	173.34
11′	171.60	171.42	172.30	172.33	173.40
12	46.13	44.77	45.41	45.34	
Ph	137.25	137.28	137.28	137.19	
Ph	128.38	128.44	128.38	128.32	
Ph	128.81	128.96	128.72	128.60	
Ph	127.44	127.56	127.38	127.32	
					17.79[c]
					17.67[d]

[a] Partial preliminary assignments based upon data and assignments for cycloheximide reported in ref 12. Carbon numbering depicted in structure **7**. [b] CD_3CN instead of $CDCl_3$ used as solvent. [c] Methyl carbon on C-2. [d] Methyl carbon on C-4.

Hydroxy ketone 7a (0.3 g): mp 129–130.5 °C; IR 3380, 1720, 1700, 1660 cm^{-1}; NMR δ 7.29 (s, 5 H), 4.94 (s, 2 H), 3.71 (br, 1 H), 2.53 (m); NMR (C_6D_6) δ 7.18 (s), 4.95 (s, 2 H), 3.45 (br, 1 H); ^{13}C NMR, see Table III. Anal. ($C_{20}H_{25}NO_4$) C, H, N.

Ketone 7b (0.2 g): mp 157.5–158 °C; IR 3420, 1715, 1700, 1675 cm^{-1}; NMR δ 7.30 (s, 5 H), 4.95 (s, 2 H), 4.18 (br, 1 H), 2.52 (m); NMR (C_6D_6) δ 7.18 (s), 4.96 (s), 3.82 (br), 3.65 (br); ^{13}C NMR, see Table III. Anal. ($C_{20}H_{25}NO_4$) C, H, N.

Diol 4f (0.14 g): mp 129–132 °C; IR 3450, 3370, 1728, 1673 cm^{-1}; NMR 7.28 (s, 5 H), 4.93 (s, 2 H), 4.23 (br, 1 H), 3.65 (br, 1 H), 2.45 (m); NMR (C_6D_6) δ 7.18 (s), 4.98 (s), 3.9 (br), 3.7 (br), 3.3 (br); ^{13}C NMR, see Table III. Anal. ($C_{20}H_{27}NO_4$) C, H, N.

Diol 4g (0.41 g): mp 115–116 °C; IR 3500, 3400, 1720, 1670 cm^{-1}; NMR δ 7.27 (s), 4.92 (s), 4.1 (br), 3.9 (br); NMR (C_6D_6) δ 7.17 (s), 4.98 (s), 3.56 (br), 3.43 (br); ^{13}C NMR, see Table III. Anal. ($C_{20}H_{27}NO_4$) C, H, N.

Pharmacological Evaluations. Testing was performed by the Antiepileptic Drug Development Program of the National Institute of Neurological and Communicative Disorders and Stroke according to established protocols.[18] In Phase I the compounds are solubilized in isotonic saline or 30% polyethylene glycol 400 and evaluated at 0.5 and 4 h after intraperitoneal injection against both maximal electroshock and subcutaneous pentylenetetrazol seizure models in mice. Each dose is evaluated in four mice for the ability to prevent tonic hindlimb extension following maximal electroshock or to prevent minimal threshold (clonic) seizures induced by the subcutaneous injection of 85 mg/kg of pentylenetetrazol. Activity is defined as the lowest dose at which at least one of four animals is protected. Compounds with activity at 100 mg/kg are further studied for quantification of median effective doses (ED_{50}) and median neurotoxic doses (TD_{50}) in Phase II studies.

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Registry No. 19, 4630-75-5; 1b, 98818-30-5; **2a**, 6276-34-2; **2b**, 363-27-9; **2c**, 92697-39-7; **2d**, 92697-40-0; **2e**, 98735-82-1; **2f**, 98735-90-1; **2h**, 98735-91-2; **3a**, 91642-30-7; **3b**, 6278-76-8; **4a**, 4624-95-7; **4b**, 98735-92-3; **4c**, 98735-81-0; **4d**, 98735-93-4; **4f**, 98735-89-8; **4g**, 98818-29-2; **4h**, 98735-96-7; **4i**, 98735-83-2; **4j**, 98735-94-5; **4k**, 98735-95-6; **4l**, 98735-84-3; **5**, 28360-59-0; **6a**, 98735-86-5; **6b**, 98735-85-4; **7a**, 98735-87-6; 7b, 98735-88-7.

Correspondence Analysis Applied to Steroid Receptor Binding

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The relative binding affinities of 48 steroids for four classes of hormone receptor (progestin, PR; androgen, AR; glucocorticoid, GR; mineralocorticoid, MR) have been analyzed by correspondence analysis. The steroids were, for the most part, derivatives of nortestosterone, differing by their degree of unsaturation, by the presence or absence of a 17α-ethynyl group, and by the length of the C-13 alkyl substituent. Derivatives of norprogesterone were included as reference compounds. Distribution maps visualizing the results of the mathematical analysis revealed that the majority of the test steroids were within the zone of influence of AR and PR and had limited affinity for GR and MR. Overall lack of specificity and enhanced affinity for GR and MR were induced by increasing unsaturation and by the presence of a C-13 ethyl group. The general and specific conclusions of the analysis confirm and extend previous intuitive and partial interpretations of the data. Correspondence analysis, however, has the advantage of taking into account the sum total of the available information, without any preconceived notion of the relative importance of a specific structural feature or biological parameter and, furthermore, enables simultaneous representation on a single graph of the receptor and steroid fields. The present example demonstrates the use of this type of methodology in processing routine screening data involving multiple parameters.

In general, one of the first steps in the determination of the potential usefulness of a molecule as a drug is screening its activity with respect to a large number of biological targets in comparison to several reference substances or structural analogues. Thus, information on the molecule is contained in a plethora of data requiring careful analysis.

This analysis can take several forms:

(a) The intrinsic complexity of the relationships among the different parameters can be partially disregarded, and the results can be analyzed methodically by, for instance, establishing maxima and minima and comparing rows and columns. The data are subjected to the rational reasoning of the experimentator whose approach, however logical, nevertheless remains intuitive.

(b) Several authors have described multiparametric models for structure–activity relationship studies with the aim of designing new drugs with preselected characteristics.[1] The common feature of their approaches is the search for the molecular descriptors (structural fragments, quantum chemical indices, physicochemical properties ...) that can discriminate among various activities.

(c) Our approach attempts to analyze the data in their overall complexity without any preconception of the particular importance, for instance, of a structural descriptor as opposed to any other feature of the molecule. The molecules position themselves relative to each other on confronting the chemical and biological fields, and the resultant configuration is analyzed. This analysis therefore does not tend toward a knowledge of the activity of the nth + 1 molecule but toward an understanding of how the molecules relate to each other with regard to the biological activities under study.

To find out how the items of the two fields are arranged in an n-dimensional system, we used factorial analysis but, in order to conserve the probabilistic nature of the system, we adopted correspondence analysis (CA) using χ^2 metrics[2] instead of principal component analysis (PCA) using a covariance matrix. The advantages of CA are at least threefold: (a) CA confers the same importance to the two fields (rows and columns), which can therefore be represented together on the same distribution map. In this way, observations can be correlated directly. (b) CA enables each representative point to be broken down into its constituent parts (principle of distribution equivalence) without affecting the factors. (c) In comparison to other factorial methods, CA does not overemphasize the elements that carry the most weight in the system. The use of this approach in the study of structure–activity relationships is licit in so far as the activity K_{ij} of molecule i in relation to biological parameter j is a function of the frequency of association between ligand and receptor. The outcome of the analysis takes the form of one or more distribution maps confronting the various items. These maps are read by the scientist in conjunction with the mathematical parameters necessary for their interpretation.

We have already used this type of approach in a few specific cases[3] but shall describe its full import in this paper by illustrating its application to the study of the

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