(Waters Associates), operated at ambient temperature, was used. The mobile phase used consisted of a mixture of acetonitrile and 0.055 M phosphate buffer, pH 6.2 (3:2). At a flow rate of 2 mL/min, **6a** has a retention time of 2 min.

Kinetics of the Disappearance of the Quaternary Salt (5a) from the Brain Homogenate. A freshly perfused Sprague–Dawley rat brain (2.0 g) was homogenized in 20 mL of phosphate buffer, pH 7.4. A solution of 10.0 mg of 1-methyl-3-(*N*-phenethylcarbamoyl)pyridinium iodide (**5a**) in 2 mL of aqueous methanol (1:1) was added, and the thoroughly mixed mixture was kept at 37 °C in a water bath. At each time period, 1 mL of the mixture was taken, shaken thoroughly with 8 mL of acetonitrile, centrifuged, and injected into the HPLC. The amount of quaternary compound in the sample was determined from a standard calibration curve and percentage recovery of a sample taken at time 0.

In Vivo Study of 1-Methyl-3-(*N*-phenethylcarbamoyl)-1,4-dihydropyridine (6a). A group of 22 male Sprague–Dawley rats of average weight of 300 ± 50 g was anesthetized with Inovar, and the freshly prepared dihydro compound (**6a**) was injected through the external jugular as a solution in Me_2SO (0.5 g/mL) at a dose of 125 mg/kg animal body weight. The amount of Me_2SO thus injected is less than 0.2 g/kg, way below the dose of 1 g/kg that was shown[25] as still *not* affecting the permeability of the blood–brain barrier. At appropriate time periods, 1 mL of blood was withdrawn from the heart and added to 3 mL of saline in a tube; then the animal was perfused with 20 mL saline solution and decapitated, and the brain was obtained. The brains were weighed and, together with the blood samples, immediately placed in the freezer for overnight storage. Each brain was homogenized in 2 mL of water; then 8 mL of acetonitrile was added, and the mixture was homogenized again and centrifuged. To the blood samples was added 16 mL of acetonitrile, and the mixture was shaken vigorously and centrifuged. The supernatants from the brain and the blood samples were analyzed in duplicate by using HPLC. The amounts of the quaternary compound were determined from a standard calibration curve, and a recovery experiment was made by injection of a certain amount of the quaternary compound directly into a freshly perfused rat brain or 1 mL of blood and then treated in the same manner as the brain and blood samples.

(25) D. M. Keane, I. Gray, and J. A. Panuska, *Cryobiology*, **14**, 592 (1977).

Acknowledgment. This research was supported by Grant GM 27167 from the National Institutes of Health.

Registry No. 4, 24303-08-0; **5a**, 84254-38-6; **5b**, 84254-40-0; **6a**, 80703-25-9; **6b**, 39713-12-7; **8a**, 84278-95-5; **8b**, 84254-42-2; **9a**, 84278-96-6; **9b**, 84254-43-3; **10a**, 84254-39-7; **10b**, 84254-41-1; nicotinoyl chloride hydrochloride, 20260-53-1; phenethylamine, 64-04-0; 5-carbethoxy-3-pyridinecarboxylic acid, 84254-37-5; trigonelline hydrochloride, 6138-41-5; phenethylamine hydrochloride, 156-28-5; diethyl 3,5-pyridinedicarboxylate, 4591-56-4; 1-methyl-3-(*N*-phenethylcarbamoyl)pyridinium, 80703-26-0.

Structure–Activity Relationships in the Antiinflammatory Steroids: A Pattern-Recognition Approach

Nicholas Bodor,* Alan J. Harget,[1] and Edward W. Phillips

Department of Medicinal Chemistry, J. H. Miller Health Center, College of Pharmacy, University of Florida, Gainesville, Florida 32610. *Received March 25, 1982*

A pattern-recognition technique has been used to determine structure–activity relationships for antiinflammatory steroids. Experimental results using the human vasoconstrictor test of McKenzie and Stoughton and the rat granuloma cotton pellet method of Meier were correlated with the various substructural descriptors. Steroids were classified into two categories according to potency, and a pattern-recognition method was applied to determine their relative ranking. The resulting structure–activity relationships obtained and the relative contributions of the various structural variables for both bioassays are discussed. A synergistic effect was predicted to be in operation between certain pairs of substituents.

The introduction and developoment of glucocorticosteroids have been the major therapeutic advances in dermatology during the past 50 years. Early work in corticosteroid research was directed to the synthesis of compounds with high antiinflammatory potency and to the reduction of side effects such as sodium retention. These attempts have been partially successful, since some corticosteroids have been synthesized that are locally active but that have reduced systemic activity.

Topical antiinflammatory activity has been enhanced by various modifications of the steroid nucleus, the most important being the removal or masking of the hydroxy groups and fluorination at the 6- and 9-positions. However, it is now believed[2] that the initial importance attached to fluorination has been overestimated, since a number of nonfluorinated steroids exhibit high potency, for example, hydrocortisone 17-butyrate and budesonide, and some fluorinated steroids display relatively low activity, such as betamethasone and dexamethasone.

In an attempt to rationalize the large volume of data that exists on the antiinflammatory steroids, we have applied a pattern-recognition technique in order to determine structure–activity relationships. Such relationships attempt to rationalize the connection between the molecular structure of a chemical compound and its measured biological activity. If such relationships could be determined for the steroids, then they would be of considerable practical and theoretical importance because of the significant role that steroids play in medicinal chemistry. For example, the relationships would allow the chemist to predict the biological activity of untested, or unsynthesized, steroids and, hence, adopt a more rational approach to drug design.

Structure–activity studies employed in medicinal chemistry in the past have used the empirical method of Hansch,[3] the mathematical model of the Free–Wilson

(1) Visiting Professor from the Computer Center, University of Aston, Birmingham, B4 7PA, England.

(2) M. D. Stewart, J. O. Runikis, S. C. Verma, and S. Wallace, *Can. Med. Assoc. J.*, **108**, 33 (1973); G. Bruini and G. Peverelli, *J. Int. Med. Res.*, **2**, 240 (1974).

(3) C. Hansch, *J. Med. Chem.*, **19**, 1 (1976); C. Hansch and T. Fujita, *J. Am. Chem. Soc.*, **86**, 1616 (1964).

0022-2623/83/1826-0318$01.50/0

approach[4], and pattern-recognition studies.[5] Despite the criticism leveled[6] at certain pattern-recognition studies for their choice of data and its representation, the pattern-recognition method, *when properly applied*, does offer the chemist useful means of determining the relationships existing in a large amount of data.

Steroids, because of their biological importance and relative molecular rigidity, represent a fruitful area for structure–activity studies. Minor molecular modifications to the steroid nucleus cause important changes in biological activity that should result in well-defined structure–activity relationships. Previous studies involving the antiinflammatory steroids have applied Hansch-type studies[7] and the use of de novo constants.[8] In an attempt to obtain more representative structure–activity relationships than those derived by previous investigators, we have considered a much greater number of steroids.

It is well known that certain corticosteroids that are predicted to be potent by animal studies are frequently found to be much less potent by human studies. The reasons for this are not known at the present time, although it might have something to do with the different metabolic transformations of the steroids in different species. Because of this discrepancy, we thought it would be instructive to compare the structure–activity relationships derived both from human and animal studies. Of the assays used, we have selected the McKenzie–Stoughton vasoconstrictor test,[9] which is a human study, and the granuloma cotton pellet method of Meier,[10] which is an animal study.

In 1962, McKenzie and Stoughton proposed a method for evaluating the antiinflammatory potency of a steroid that in its original and modified forms has come to be widely accepted. In its original form, an alcoholic solution of the steroid is applied to different sites on the human forearm. After evaporation of the alcohol, the area is occluded for 16 h and then the skin evaluated for relative vasoconstriction (blanching). Because of the variable vasoconstriction response, the method requires a large number of human subjects. Despite the seemingly imprecise nature of the assay, the method gives a good indication of the clinical activity of a drug.[11] The assay measures the combined effect of a number of features, for example, the ability of the steroid to penetrate the skin barrier, its intrinsic activity at and subsequent clearance from the reaction site, local metabolism, binding, etc.

An extensive literature survey was undertaken to determine the vasoconstrictor activity of corticosteroids as measured by the McKenzie–Stoughton method or one of its derived methods. After the elimination of those structures, which contained unique substituents, 122 steroids remained. The elimination process was necessary if meaningful structure–activity relationships were to be obtained. The remaining steroids were classified into two categories, potent (P) and nonpotent (NP), the classification being determined relative to the potency of hydrocortisone 17-butyrate, which was selected arbitrarily as the standard corticosteroid. Of the 122 steroids considered, 74 were classified as potent and 48 as nonpotent.

In the cotton pellet granuloma test of Meier, the granuloma tissue is produced by implanting a cotton pellet of known weight into a subcutaneous pocket in rats. The degree of inflammation can be measured by the accumulation of granuloma tissue around the pellet, and the antiinflammatory activity of the drug can be determined by the inhibition of granuloma tissue. Drugs can be administered systemically by oral or subcutaneous administration, or locally by direct absorption into the pellet. Several modifications have been proposed[12] to the cotton pellet method in order to improve the precision of the assay. The granuloma pouch technique,[13] which is similar to the cotton pellet method, was initially considered as a likely candidate for the animal study, but it was rejected because it appears that the accuracy of this assay is questionable and its effectiveness for granulomatous reaction has been criticized.[14] Table II lists the steroids and their potencies in these assays following systemic administration.

After elimination of structures that contain unique substituents, the remaining 78 steroids were classified into two categories of approximately equal size by assigning steroids containing a potency greater than 15 to the potent category (31 steroids) and the remainder to the nonpotent category (47 steroids).

The Pattern-Recognition Analysis Used. During the past 10 years, a number of papers have appeared describing chemical applications of pattern recognition. The usefulness of the technique has been tested in such diverse areas as the analysis of mass spectra,[15] assistance in material production problems,[16] and the determination of pharmacological activity.[17] Pattern recognition is useful for dealing with data of high dimensionality where the deduction of relationships is difficult. Although the technique is empirical, it is capable, when properly used, of providing the experimentalist with some insight into the relationships contained within the experimental data. The sole assumption made by the technique is that a relationship exists between the observed experimental data and the defined categories, although even this assumption will be investigated by the technique.

In the present study, we have applied a pattern-recognition technique, the linear learning machine method, in an attempt to develop classification rules capable of distinguishing between potent and nonpotent steroids. Essentially, a training set was composed of a number of steroids of known activity and classified according to potency into one of two categories. The linear learning machine method was then applied in an attempt to create a linear decision surface that would be capable of separating the potent steroid of the training set from the nonpotent ones. The computer programs were written and

(4) S. M. Free and J. W. Wilson, *J. Med. Chem.*, **78** 395 (1964).
(5) P. C. Jurs and T. L. Isenhour, "Chemical Applications of Pattern Recognition", Wiley, New York, 1975.
(6) C. L. Perrin, *Science*, **183**, 551 (1974); J. T. Clerc, P. Naegeli, and J. Seibl, *Chimia*, **27**, 639 (1973).
(7) M. E. Wolff and C. Hansch, *Experimentia*, **29**, 1111 (1973); R. A. Coburn and A. J. Solo, *J. Med. Chem.*, **19**, 748 (1976); P. Ahmad and A. Mollers, *J. Steroid Biochem.*, **7**, 19 (1976).
(8) J. Fried and A. Borman, *Vitam. Horm. (N.Y.)* **16**, 303 (1958).
(9) A. W. McKenzie and R. B. Stoughton, *Arch. Dermatol.*, **86**, 608 (1962).
(10) R. Meier, W. Schuler, and P. Desarilles, *Experientia*, **6**, 469 (1950).
(11) J. Reid and D. B. Brookes, *Br. J. Dermatol.*, **80**, 328 (1968); K. H. Burdick, *Acta. Derm. Venerol. Suppl.*, **52** (67), 19 (1972); B. W. Barry and R. Woodford, *Brit. J. Dermatol.*, **97**, 555 (1977).
(12) V. Robinson and J. M. Robson, *Br. J., Pharmacol.*, **23**, 420 (1964); G. Benzi and G. M. Frigo, *Farmaco, Ed. Prat.*, **19**, 327 (1964); K. F. Swingle and F. E. Shideman, *J. Pharmacol.*, **183**, 226 (1972).
(13) H. Selye, *JAMA, J. Am. Med. Assoc.*, **152**, 1207 (1953); A. Robert and J. E. Nezamis, *Acta. Endocrinol. (Copenhagen)*, **25**, 105 (1957).
(14) M. W. Whitehouse, *Prog. Drug Res.*, 8 (1965).
(15) P. C. Jurs, B. R. Kowalski, T. L. ISenhour, and C. N. Reilley, *Anal. Chem.*, **42**, 1387 (1970).
(16) B. R. Kowalski, *CHEMTECH*, 300 (1974).
(17) K. Yuta and P. C. Jurs, *J. Med. Chem.*, **24**, 241 (1981).

Table I. Experimental Vasoconstrictor Activity of the Steroids Studied

no.	compound	structural features present[a]					potency: exptl[b]	potency: classified[c]	ref[b]
1	clobetasol 17-propionate	3	4	8	20		1869	P	*d, l*
2	dexamethasone	3	5	6	15		1.0; 0.8	NP	*e, g*
3	beclomethasone 17, 21-dipropionate	4	8	17			500	P	*g, h*
4	hydrocortisone 21-acetate	1	6	16			1.0	NP	*e, f*
5	fluocinolone acetonide	2	3	15	22		100	P	*e*
6	hydrocortisone	1	6	15			<1	NP	*e*
7	9α-fluorohydrocortisone	1	3	6	15		0.1	NP	*e*
8	prednisolone 21-acetate	6	16				1.0	NP	*e*
9	halcinonide	1	3	20	22		>360	P	*i, s*
10	9α-fluoro-21-chloro-11β-hydroxy-16β-methyl pregna-1,4-diene-3,20-dione 17-butyrate	3	4	9	20		1166	P	*l*
11	corticosterone	1	15				0.3	NP	*g, aa*
12	prednisolone	6	15				0.1	NP	*e, i*
13	hydrocortisone 17-butyrate	1	9	15			50	P	*l, m*
14	fluocinonide	2	3	16	22		~300; 500	P	*i, n*
15	ethyl 17α-acetoxy-9-fluoro-11β-hydroxy-16β-methyl-3-oxoandrosta-1,4-diene-17β-carboxylate	3	4	7	12		16	NP	*l*
16	propyl 9-fluoro-11β,17α-dihydroxy-16β-methyl-3-oxoandrosta-1,4-diene-17β-carboxylate	3	4	6	13		5	NP	*l*
17	11β-hydroxy-16α,17α-dihydroxy-16β-methyl-pregna-1,4-diene-3,20-dione 21-acetate	5	16				NA	P	*o*
18	diflucortolone 21-valerate	2	3	5			NA	P	*p*
19	fluocortolone	2	5	15			NA	P	*bb*
20	flumethasone 21-pivalate	2	3	5	6		360	P	*f, l*
21	dexamethasone 21-phosphate	3	5	6			1-2	NP	*e*
22	flurandrenolone	2	6	15			12; 10	NP	*e, i*
23	flurandrenolone acetonide	2	15	22			100-300	P	*i*
24	triamcinolone acetonide	3	5	12			100	P	*e, f*
25	betamethasone 17-isobutyrate	3	4	15			470	P	*l*
26	betamethasone 17-valerate	3	4	10	15		360	P	*f, ff*
27	betamethasone 21-acetate	3	4	6	16		18; 18-33	NP	*i, ff*
28	betamethasone	3	4	6	15		0.8	NP	*f, n*
29	methyl prednisoloneacetate	6	16				1	NP	*e*
30	9α-fluoro-16α,17-dimethylpregna-1,4-diene-3,20-dione 21-propionate	3	5	17			NA	P	*o*
31	methylthiomethyl 17α-(pentanoyloxy)-11β-hydroxy-3-oxoandrost-4-ene-17β-carboxylate	1	10	14			NA	NP	*r*
32	9α-fluoro-21-chloro-11β,16α,17α-trihydroxypregna-1,4-diene-3,20-dione 16,17-acetonide	3	20	22			NA	P	*s*
33	9α-fluoro-11β,17α-dihydroxy-16β-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid	3	4	6	11		<1	NP	*l*
34	methyl 17α-acetoxy-9α-fluoro-11β-hydroxy-16β-methyl-3-oxoandrost-1,4-diene-17β-carboxylate	3	4	7			385	P	*l*
35	betamethasone 21-isobutyrate	3	4	6	19		90	P	*l*
36	chloromethyl 17α-(propanoyloxy)-9-fluoro-11β-hydroxy-16β-methyl-3-oxoandrosta-1,4-diene-17β-carboxylate	3	4	8			400	P	*l*
37	paramethasone	2	5	6	15		NA	NP	*t*
38	betamethasone 21-butyrate	3	4	6	18		85	P	*ff*
39	chloromethyl 17α-(propanoyloxy)-11β-hydroxy-3,20-dioxopregn-4-en-21-oate	1	8	21			NA	P	*r*
40	11β-hydroxy-16α,17α,21-trimethylpregna-1,4-diene-3,20-dione	5					NA	P	*o, x*
41	methyl 17α-(butanoyloxy)-9-fluoro-11β-hydroxy-16β-methyl-3-oxandrosta-1,4-diene-17β-carboxylate	3	4	9			175	P	*l*
42	betamethasone 21-valerate	3	4	6			26	NP	*ee, ff*
43	betamethasone 17-acetate	3	4	7	15		114	P	*ff*
44	6α,9α-difluoroprednisolone 17-isobutyrate	2	3	15			<360	P	*p*
45	6α,9α-difluorohydrocortisone 17-valerate	1	2	3	10	15	360	P	*p*
46	6α,9α-difluoroprednisolone 17-propionate	2	3	8	15		<360	P	*p*
47	6α,9α-difluoroprednisolone 17,21-dibutyrate	2	3	9	18		540	P	*p*
48	triamcinolone	3	6	15			0.01	NP	*e, g*
49	dexamethasone 21-acetate	3	5	6	16		2-3; 8	NP	*i, m*
50	6α,9α-difluoroprednisolone 17-propionate 21-trimethylacetate	2	3	8			720	P	*u*
51	prednisolone 17-valerate	10	15				<1	NP	*i*
52	ethyl 17α-(propanoyloxy)-9-fluoro-11β-hydroxy-16β-methyl-3-oxoandrosta-1,4-diene-17β-carboxylate	3	4	8	12		75	P	*l*
53	6α,9α-difluoro-21-deoxyprednisolone 17-acetate	2	3	7			540	P	*v*
54	beclomethasone	4	6	15			0.8	NP	*g*
55	paramethasone 21-acetate	2	5	6	16		NA	P	*t*
56	6α,9α-difluoroprednisolone 17,21-diacetate	2	3	7	16		360	P	*u*

Table I (*Continued*)

no.	compound	structural features present[a]					potency: exptl[b]	potency: classified[c]	ref[b]
57	9α-fluoro-21-chloro-11β-hydroxy-16β-methyl-pregna-1,4-diene-3,20-dione 17-isobutyrate	3	4	20			378	P	*l*
58	prednisolone phosphate	6					0.1	NP	*e*
59	propyl 17α-(propanoyloxy)-9-fluoro-11β-hydroxy-16β-methyl-3-oxoandrosta-1,4-diene-17β-carboxylate	3	4	8	13		30	NP	*l*
60	17α-acetoxy-9-fluoro-11β-hydroxy-16β-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid	3	4	7	11		<1	NP	*l*
61	methyl 17α-(propanoyloxy)-9-fluoro-11β-hydroxy-16β-methyl-3-oxoandrosta-1,4-diene-17β-carboxylate	3	4	8			390	P	*l*
62	betamethasone 17-butyrate	3	4	9	15		168	P	*ff*
63	betamethasone 21-propionate	3	4	6	17		40	NP	*l*
64	hydrocortisone 17-valerate	1	10	15			16–34	NP	*i, m*
65	chlorcortolone	2	5	15			NA	P	*dd*
66	desonide	15	22				NA	P	*i, z*
67	9α-fluorohydrocortisone acetate	1	3	6	16		1.0	NP	*e*
68	methyl prednisolone	6	15				0.1	NP	*e*
69	methylthiomethyl 11β,17α-dihydroxy-3-oxoandrost-4-ene-17β-carboxylate	1	6	14			NA	NP	*g*
70	chloromethyl 17α-(propanoyloxy)-11β-hydroxy-3-oxoandrost-4-ene-17β-carboxylate	1	8				NA	P	*g*
71	9α-fluoro-11β,21-dihydroxy-16α,17α-dimethyl-pregna-1,4-diene-3,20-dione	3	5	15			NA	P	*o*
72	chloromethyl 6α,9α-difluoro-11β-hydroxy-3,20-dioxopregna-1,4-diene-21-oate 16,17-acetonide	2	3	21	22		NA	P	*g*
73	17α-(propanoyloxy)-9-fluoro-11β-hydroxy-16β-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid	3	4	8	11		<1	NP	*l*
74	6α,9α-difluoroprednisolone 17-isobutyrate 21-acetate	2	3	16			~720	P	*u*
75	6α,9α-difluoroprednisolone 17-acetate 21-isobutyrate	2	3	7	19		~720	P	*u*
76	6α,9α-difluoroprednisolone 17-acetate	2	3	7	15		<360	P	*u*
77	6α,9α-difluoroprednisolone	2	3	6	15		<360	NP	*u*
78	6α,9α-difluoro-11β-hydroxypregn-4-ene-3,20-dione 17-valerate 21-acetate	1	2	3	10	16	~360	P	*u*
79	ethyl 9-fluoro-11β-hydroxy-16β-methyl-3-oxo-17α-(butanoyloxy)androsta-1,4-diene-17β-carboxylate	3	4	9	12		40	NP	*l*
80	deoxymethasone	3	5	15			NA	P	*j*
81	21-deoxycortisol	1	6				0.4	NP	*aa*
82	betamethasone 17,21-dipropionate	3	4	8	17		1300	P	*bb*
83	propyl 9-fluoro-11β-hydroxy-16β-methyl-3-oxy-17α-(butanoyloxy)androsta-1,4-diene-17β-carboxylate	3	4	9	13		40	NP	*l*
84	21-deoxybetamethasone 17-propionate	3	4	8			1170	P	*cc*
85	6α,9α-difluoroprednisolone 17-acetate 21-butyrate	2	3	7	18		NA	P	*u*
86	6α,9α-difluoroprednisolone 17-propionate 21-isobutyrate	2	3	8	19		1080	P	*u*
87	difluocortolone trimethylacetate	2	3	5			NA	P	*dd*
88	6α,9α-difluoroprednisolone 17-valerate	2	3	10	15		360	P	*u*
89	6α,9α-difluoroprednisolone 17-butyrate	2	3	9	15		<360	P	*u*
90	6α,9α-difluoro-21-deoxyprednisolone 17-propionate	2	3	8			900–1080	P	*v*
91	fluprednisolone	2	6	15			1	NP	*i*
92	6α,9α-difluoroprednisolone 17-valerate 21-acetate	2	3	10	16		720	P	*u, ee*
93	flurandrenolone acetate	2	6	16			6–8	NP	*i*
94	methyl 9-fluoro-11β,17α-dihydroxy-16β-methyl-3-oxoandrostra-1,4-diene-17β-carboxylate	3	4	6			<1	NP	*l*
95	9-fluoro-11β-hydroxy-16β-methyl-3-oxo-17α-(pentanoyloxy)androsta-1,4-diene-17β-carboxylic acid	3	4	10	11		<1	NP	*l*
96	21-chloro-11β,16α,17α-trihydroxypregna-1,4-diene-3,20-dione 16,17-acetonide	20	22				NA	P	*s*
97	6α,9α-difluoroprednisolone 17-butyrate 21-acetate	2	3	9	16		1170	P	*u, ee*
98	6α,9α-difluoroprednisolone 17-butyrate 21-propionate	2	3	9	17		1058	P	*u, ee*
99	hydrocortisone 17-acetate	1	7	15			NA	NP	*m*
100	medrysone	1					<1	NP	*i*
101	chloromethyl 11β,17α-dihydroxy-3-oxoandrost-4-ene-17β-carboxylate	1	6				NA	NP	*r*
102	betamethasone 17-propionate	3	4	8	15		190	P	*ff*
103	hydrocortisone phosphate	1	6				0.01	NP	*e*

Table I (*Continued*)

no.	compound	structural features present[a]				potency: exptl[b]	potency: classified[c]	ref[b]
104	methylthiomethyl 9α-fluoro-17α-(pentanoyloxy)-16β-methyl-11β-hydroxy-3-oxoandrosta-1,4-diene-17β-carboxylate	3	4	10	14	NA	NP	*r*
105	6α,9α-difluoroprednisolone 17,21-dipropionate	2	3	8	17	853	P	*u, ee*
106	methyl 17α-(pentanoyloxy)-9-fluoro-11β-hydroxy-16β-methyl-3-oxoandrosta-1,4-diene-17β-carboxylate	3	4	10		7	NP	*l*
107	fluocinolone	2	3	6	15	15-45	NP	*i, n*
108	6α,9α-difluoroprednisolone 17-propionate 21-acetate	2	3	8	16	1080	P	*u*
109	21-chloro-11β,16α,17α-trihydroxypregn-4-ene-3,20-dione 16,17-acetonide	1	20	22		NA	P	*s*
110	9α-fluoro-21-chloro-11β,17α-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione	3	4	6	20	NA	NP	*l*
111	6α,9α-difluoroprednisolone 17-butyrate 21-isobutyrate	2	3	9	19	1080	P	*u*
112	6α,9α-difluoroprednisolone 17-propionate 21-butyrate	2	3	8	18	1260	P	*u*
113	beclomethasone 17-propionate	4	8	15		360	P	*g*
114	6α,9α-difluorodeoxyprednisolone 17-butyrate	2	3	9		900-1080	P	*v*
115	desfluochlorcortolone trimethylacetate	5				NA	P	*dd*
116	chloromethyl 17α-(pentanoyloxy)-11β-hydroxy-3,20-dioxopregn-4-en-21-oate	1	10	21		NA	P	*r*
117	prednisolone 17-valerate 21-acetate	10	16			NA	P	*y*
118	6α,9α-difluoroprednisolone 17-isobutyrate 21-propionate	2	3	17		900	P	*u*
119	6α,9α-difluoroprednisolone 17,21-diisobutyrate	2	3	19		720	P	*u*
120	betamethasone phosphate	3	4	6		NA	NP	*h*
121	9α-fluoro-21-chloro-11β-hydroxy-16β-methyl-pregna-1,4-diene-3,20-dione 17-valerate	3	4	10	20	91	P	*l*
122	9α-fluoro-21-chloro-11β-hydroxy-16β-methyl-pregna-1,4-diene-3,20-dione 17-acetate	3	4	7	20	812	P	*l*

[a] Numbering of substituents according to subsequent Table III. [b] Standard McKenzie-Stoughton test. Activity relative to fluocinolone 16,17-acetonide. [c] Classification according to the present study, potent (P) or nonpotent (NP), relative to hydrocortisone 17α-butyrate. [d] C. G. Sparkes and L. Wilson, *Br. J. Dermatol.*, 90, 197 (1974). [e] A. W. McKenzie, *Arch. Dermatol.*, 86, 611 (1962). [f] K. J. Child, A. F. English, H. G. Gilbert, A. Hewitt, and E. A. Woollett, *Arch. Dermatol.*, 97, 407 (1968). [g] D. M. Harris, *J. Steroid Biochem.*, 6, 711 (1975). [h] L. Wilson, *Br. J. Dermatol.*, 94 (suppl 12), 33 (1976). [i] O. J. Lorenzetti, *Curr. Ther. Res.*, 25, 92 (1979). [j] R. Cornell, *Pharm. Ther.*, 11, 47 (1980). [k] T. L. Popper and A. S. Watnick, "Antiinflammatory Agents, Chemistry and Pharmacology", R. A. Scherrer and M. W. Whitehouse, Eds., 1974. [l] G. H. Phillips, "Mechanism of Topical Corticosteroid Activity", L. Wilson and R. Marks, Eds., Churchill Livingstone, New York, 1976. [m] D. J. C. Engel, A. F. Marx, R. F. Rekker, and L. van Wyk, *Arch. Dermatol.*, 109, 863 (1974). [n] R. B. Stoughton, *Arch. Dermatol.*, 99, 753 (1969). [o] B. W. Barry and A. R. Brace, *J. Inves. Dermatol.*, 64, 418 (1975). [p] G. L. Coleman, I. Kanfer, and J. M. Haigh, *Dermatologica*, 156, 224 (1978). [q] Footnote omitted on revision. [r] N. Bodor and R. Little, personal communication. [s] F. K. Bagatell and M. A. Augustine, *Curr. Ther. Res.*, 16, 748 (1974). [t] V. A. Place, J. G. Velazquez, and K. H. Burdick, *Arch. Dermatol.*, 101, 531 (1970). [u] R. Gardi, R. Vitali, G. Falconi, and A. Ercoli, *J. Med. Chem.*, 15, 556 (1972). [v] R. Vitali, S. Gladiali, G. Falconi, G. Celasco, and R. Gardi, *J. Med. Chem.*, 20, 853 (1977). [w] Footnote omitted on revision. [x] A. J. Lewis and P. K. Fox, *J. Pharm. Pharmacol.*, 28 (suppl), 82P (1976). [y] G. Bruni and G. Peverelli, *J. Int. Med. Res.*, 2, 240 (1974). [z] M. D. Steward, J. O. Rumkis, S. C. Verma, and S. Wallace, *Can. Med. Assoc. J.*, 108, 33 (1973). [aa] P. M. Sutton, R. J. Feldmann, and H. I. Maibach, *J. Inves. Dermatol.*, 57, 371 (1971). [bb] V. Voightlander, *J. Int. Med. Res.*, 5, 128 (1977). [cc] M. J. Bussee, P. Hunt, K. A. Lees, P. N. D. Maggs, and T. M. McCarthy, *Br. J. Dermatol.*, 81 (suppl 4), 103 (1969). [dd] H. Baker and H. A. Sattar, *Br. J. Dermatol.*, 80, 46 (1968). [ee] G. Falconi and G. L. Rossi, *Arch. Dermatol.*, 105, 856 (1972). [ff] A. W. McKenzie and R. M. Atkinson, *Arch. Dermatol.*, 89, 741 (1964).

developed by the authors in PASCAL for use on the University of Florida Amdahl 470 V/6-II system.

The linear learning machine method has been widely discussed in the literature,[18,19] so we shall present only the essential elements of the method here.

The experimental data to be classified is represented by a pattern vector of the form:

$$X = X_1, X_2, X_3, \cdots, X_n$$

where each element, X_1, of the pattern vector represents a physically measurable quantity. The dimensionality, n, of the vector indicates the number of features, or observations, necessary to describe the pattern. If each compound in the training set is represented by a point in n-dimensional space, it may be expected that compounds of similar biological activity would lie in one limited region of the space and be separated from compounds of different biological potency from the remainder.

Linear separable data can be separated by a linear discriminant function of the form:

$$S = \sum_{i=1}^{n+1} w_i X_i$$

where X_i is an element of the pattern vector, and w_i is the element of the weight vector associated with S_i. A $n + 1$ component is added where $X_{n+1} = 1$, so that the category is determined by the sign of the dot product, S; that is, $S > 0$ implies category 1 and $S < 0$ implies category 2 in the area of the pattern classifier, the effect of which is to maximize the separation of the two categories. In this case, classification using the dot product proceeds according to

(18) N. J. Nilsson, "Learning Machines", McGraw-Hill, New York, NY, 1965.
(19) T. L. Isenhour and P. C. Jurs, *Anal. Chem.*, 43 (10), 20A (1971).

the following relationships: $S > 0$ implies category 1; $S < 0$ implies category 2.

The main problem is to determine the weight vector, i.e., the set of weights (w_1, w_2, ..., w_{n+1}), such that each member of the training set is assigned to the correct category according to the relationships given above. The weight vector is determined by an error-correction feedback algorithm. One popular algorithm, and the one used in this study, modifies the weight vector such that the linear decision surface is reflected about the misclassified point; i.e., the dot product S has the same magnitude but the opposite, and therefore correct, sign. This algorithm guarantees convergence if the data are linearly separable, although the rate of convergence cannot be predicted. The sole criterion for convergence is the correct classification of all members of the training set. If convergence is not obtained, the training process terminates after a predetermined number of iterations.

Since the approach is an empirical one and not constrained by theory, relationships may be determined from the weight vectors that may not have otherwise been considered. Furthermore, convergence in the training phase may allow the potency of unsynthesized, or untested, steroids to be predicted with some confidence. The predictive power of the method is enhanced if a large and representative training set is taken.

The application of the linear learning machine method to the antiinflammatory steroids and the results obtained are discussed in detail below. He LLM method was adopted instead of a quantitative prediction of potency of steroids, because of the known experimental inaccuracies of the bioassays. In many cases, quantitative measure of potency to a steroid is not available, and when they are, the figures were found to vary between different laboratories, or cover a range of values.

Results and Discussion

The computational description of molecular structure is a major problem in chemical pattern-recognition studies. At the present time, we believe there is not satisfactory means of describing chemical compounds of diverse structure for pattern-recognition requirements. In this study we have considered compounds of similar structure for which computational description is facilitated.

The relatively rigid structure of the steroid nucleus allows the structure of each steroid to be unambiguously described by employing substructural descriptors that are used to indicate the presence or absence of the associated substructure. In the case of the vasoconstrictor study, it was found that the 122 steroids could be described by a total of 33 descriptors. In order that meaningful structure–activity relationships could be determined, nonessential descriptors were eliminated by the weight-sign change feature selection technique.[20] By using this technique, a descriptor was retained if the sign of its weight vector component was found to be invariant to the initial weight value taken for the learning machine. This procedure reduced the number of descriptors from 33 to 22. In the granuloma study, 17 descriptors were originally required to describe the 78 steroids for which granuloma information was available. By using the weight-sign change feature selection technique, the 17 descriptors were reduced to 13. The descriptors and their number of occurrences are listed in Table III.

A requirement of the linear learning machine method is that the number of compounds in the training set should exceed, by at least a factor of 3, the number of descriptors if chance separation is to be avoided.[21] A further requirement states that the population of the least populated category should be greater than the number of descriptors.[22] Both of these conditions were fulfilled by the vasoconstrictor and granuloma data sets; hence, the resulting weight vectors obtained for both of these studies must be considered meaningful. The covariance matrices were found to be approximately equal for the classes. The results show that the data are, to all intents and purposes, statistically independent.

The linear learning machine method was applied to the vasoconstrictor and granuloma training sets to determine those weight vectors that would correctly classify each steroid according to the potencies given in Tables I and II. Complete convergence was obtained in the training procedure for each study. The resulting weight vectors obtained for both sets of data are shown in Table IV. The magnitude of the weight vector may give some indication of the contribution the feature makes to the classification, while the sign of the weight vector indicates whether the contribution enhances potency (given by positive values) or decreases potency (given by negative values). If sufficiently large numbers of combinations of the various substituents (structural descriptors) are used, a relative quantitative ranking of the contribution of the descriptors to the potency can be obtained. A proof for this can be provided by plotting the sum of the descriptor contributions against the measured biological activities. In the present cases, the granuloma studies should give better correlations due to the more quantative nature of the test. If we first consider the weight vectors obtained for the vasocontrictor study, Table IV, it can be seen that there is general agreement between the results and experiment. For example, the 6-fluoro, 9-fluoro, and 16,17-acetonide groupings are predicted to increase potency. It must be remembered that the weight vectors are *relative* values; that is to say, for example, that replacement of an ester group in the 17-position by an hydroxy group will lead to a decrease in potency but not necessarily to a nonpotent steroid, potency here being measured *relative* to that of hydrocortisone 17-butyrate.

For a compound to be an effective topical antiinflammatory agent, it must remain in the epidermis and migrate only slowly into the dermis (vasoconstrictor assay). This property is enhanced by the conversion of hydroxy groups in the steroid to more lipophilic derivatives. The results shown in Table IV confirm this, since most of the lipophilic groups, like the esters, are assigned positive values. For the 17α-ester groups, the results show a similar parabolic dependence with activity as that found by Wieriks.[23] Although our results predict 17α-propionate to be the most potent of the 17α-ester substituents, in contrast to that of 17α-butyrate found by Wieriks, our results were derived from a much larger set of steroids and are therefore considered to be more representative.

The method predicts 21-butyrate and 21-isobutyrate to be the most potent of the substituents studied. We believe their importance has been overemphasized, however, for the following reason. Betamethasone 21-butyrate and betamethasone 21-isobutyrate have potencies, measured[24]

(20) T. L. Isenhour, B. R. Kowalski, and P. C. Jurs, *CRC Crit. Rev., Anal. Chem.*, 4, 1 (1974).

(21) C. F. Bender, H. D. Shepherd, and B. R. Kowalski, *Anal. Chem.*, **45**, 617 (1973); D. H. Foley, *IEEE Trans. Inf. Theory*, **IT-18**, 618 (1972).

(22) E. K. Whalen-Pedersen and P. C. Jurs, *J. Chem. Inf. Comput. Sci.*, **19**, 264 (1979).

(23) J. Wieriks, W. Hespe, K. D. Jaitly, and B. L. van Kan, *Dermatologica*, **152**, 181 (1976) (suppl 1).

Table II. Experimental Relative Rat Granuloma Activity of Selected Steroids

no.	compound	structural features present[a]	potency: exptl[b]	potency: classified[c]	ref[b]
1	dexamethasone	3 9 10 11	200; 150	P	*d, e*
2	hydrocortisone 21-acetate	1 10 12	1.0	NP	*i*
3	fluocinolone acetonide	2 3 11 13	450; ~100	P	*i, u*
4	hydrocortisone	1 10 11	1	NP	*d*
5	9α-fluorohydrocortisone	1 3 10 11	6	NP	*e*
6	prednisolone 21-acetate	10 12	3	NP	*i, j*
7	fluocortolone	2 9 11	350	P	*y*
8	corticosterone	1 11	0.3	NP	*s, x*
9	triamcinolone acetonide	3 11 13	49	P	*t*
10	prednisolone	10 11	3; 4	NP	*e, s*
11	betamethasone	3 8 10 11	35.8; 70	P	*f, h*
12	hydrocortisone 17-butyrate	1 11	1	NP	*z*
13	paramethasone	2 9 10 11	63.6	P	*i*
14	triamcinolone	3 7 10 11	2.6; 5	NP	*g, i*
15	paramethasone 21-acetate	2 9 10 12	60	P	*l*
16	9α-fluorohydrocortisone 21-acetate	1 3 10 12	7.3; 13	NP	*j, x*
17	methylprednisolone	10 11	5; 6	NP	*j, i*
18	desonide	11 13	66.2	P	*aa*
19	cortisone	1 6 10 11	0.4	NP	*u*
20	deoxymethasone	3 9 11	~10; 15	NP	*v, y*
21	fluocinolone	2 3 7 10 11	19.7; 35	P	*g, i*
22	16α-methylhydrocortisone	1 9 10 11	1.4; 3.0	NP	*e, r*
23	16β-methylprednisolone	8 10 11	23; 24	P	*d*
24	16β-methylhydrocortisone	1 8 10 11	4	NP	*d*
25	16α-methylprednisolone	9 10 11	11.3	NP	*d, r*
26	16α-methyl-9α-fluorohydrocortisone	1 3 9 10 11	36	P	*e*
27	9α-fluoroprednisolone	3 10 11	17.7	P	*e, i*
28	16α-methylprednisone	6 9 10 11	13	NP	*e*
29	16β-methylprednisone	6 8 10 11	26	P	*f*
30	16β-methylcortisone	1 6 8 10 11	2	NP	*f*
31	16β-methyl-9α-fluorohydrocortisone	1 3 8 10 11	23	P	*f*
32	6α-fluoro-16α-hydroxyhydrocortisone	1 2 7 10 11	5	NP	*g*
33	6α-fluoro-16α-hydroxyhydrocortisone 16,17-acetonide 21-acetate	1 2 7 12 13	4	NP	*g*
34	6α-fluoro-16α-hydroxyprednisolone 16,17-acetonide 21-acetate	2 7 12 13	20	P	*g*
35	6α,9α-difluoro-16α-hydroxyhydrocortisone	1 2 3 7 10 11	15	P	*g*
36	9α-chloro-16α-methylprednisolone	5 9 10 11	60	P	*h*
37	6α-fluorodexamethasone	2 3 9 10 11	200-500	P	*h*
38	cortisone acetate	1 6 10 12	0.3; 0.5	NP	*i, x*
39	6α-methyldexamethasone	3 9 10 11	200	P	*h*
40	prednisone acetate	6 10 12	1.0	NP	*i*
41	9α-fluoroprednisolone acetate	3 10 12	14.0	NP	*j*
42	corticosterone acetate	1 12	0.3	NP	*k*
43	11-dehydrodexamethasone	3 6 9 10 11	140	P	*h*
44	11-dehydrocorticosterone 21-acetate	1 6 12	0.2	NP	*k*
45	6α,9α-difluoro-16α-hydroxy-1,2-dihydroprednisolone 16,17-acetonide	1 2 3 11 13	103	P	*i*
46	9α-fluorocorticosterone 21-acetate	1 3 12	2.7; 3.5	NP	*k, x*
47	6α,9α-difluoro-16α-methylhydrocortisone 21-acetate	1 2 3 9 10 12	50; 65	P	*l, m*
48	6α-fluoro-16α-methylhydrocortisone acetate	1 2 9 10 12	5	NP	*m*
49	6α,9α-difluoro-16α-methylprednisolone 21-acetate	2 3 9 10 12	120; 300	P	*l, m*
50	11β-chloro-17α-hydroxypregna-1,4-diene-3,20-dione 21-acetate	10 12	0.15-0.5	NP	*n*
51	6α,9α-difluorohydrocortisone 21-acetate	1 2 3 10 12	50; 100	P	*m, o*
52	9α,11β-dichloro-17α-hydroxypregn-4-ene-3,20-dione 21-acetate	1 5 10 12	0.15-0.5	NP	*n, p*
53	6α,9α-difluoroprednisolone 21-acetate	2 3 10 12	200	P	*m, o*
54	6α-fluorocortisone 21-acetate	1 2 6 10 12	10	NP	*o*
55	6α-fluoroprednisone 21-acetate	2 6 10 12	20	P	*o*
56	6α-fluorohydrocortisone 21-acetate	1 2 10 12	10	NP	*o*
57	6α-fluoroprednisolone 21-acetate	2 10 12	20	P	*o*
58	9α,11β-dichloro-17α-hydroxypregna-1,4-diene-3,20-dione 21-acetate	5 10 12	0.5	NP	*p*
59	16α-methyl-11β,21-dihydroxypregna-1,4-diene-3,20-dione	9 11	3	NP	*s, y*
60	9α-fluorocortisone 21-acetate	1 3 6 10 12	10	NP	*x*
61	9α-chlorohydrocortisone 21-acetate	1 5 10 12	3.5	NP	*x*
62	2α-methylhydrocortisone	1 10 11	3.5	NP	*k*

Table II (*Continued*)

no.	compound	structural features present[a]	potency: exptl[b]	potency: classified[c]	ref[b]
63	2α-methyl-9α-fluorohydrocortisone 21-acetate	1 3 10 12	9	NP	*k, dd*
64	2α-methylcortisone	1 6 10 11	<0.07	NP	*k*
65	2α-methyl-11-dehydrocorticosterone 21-acetate	1 6 12	<0.01	NP	*k*
66	2α-methylcorticosterone 21-acetate	1 12	0.3	NP	*k*
67	2α-methyl-9α-fluorocorticosterone 21-acetate	1 3 12	2.4	NP	*k*
68	6α,9α-difluoroprednisolone 17-butyrate 21-acetate	2 3 12	~200	P	*bb*
69	6α-chlorocortisone 21-acetate	1 4 6 10 12	1	NP	*cc*
70	6α-chloro-9α-fluorocortisone 21-acetate	1 3 4 6 10 12	8	NP	*cc*
71	6α-chloroprednisone 21-acetate	4 6 10 12	4	NP	*cc*
72	6α-chlorohydrocortisone 21-acetate	1 4 10 12	11	NP	*cc*
73	6α-chloro-9α-fluorohydrocortisone 21-acetate	1 3 4 10 12	11	NP	*cc*
74	6α-chloro-16-hydroxy-9α-fluoro-hydrocortisone	1 3 4 11 13	50	P	*cc*
75	6α-chloroprednisolone	4 10 11	14	NP	*cc*
76	6α-chloro-9α-fluoroprednisolone 21-acetate	3 4 10 12	27	P	*cc*
77	6α-chloro-16-hydroxy-9α-fluoroprednisolone	3 4 11 13	400	P	*cc*
78	2α-methylhydrocortisone 21-acetate	1 10 12	4.5	NP	*dd*

[a] See footnote *a* of Table I. [b] Relative to hydrocortisone and hydrocortisone 21-acetate, both having potency = 1. [c] Compounds with values higher than 15 were classified potent (P); compounds with values lower than 15 were classified as nonpotent (NP). [d] R. H. Silber, *Ann. N. Y. Acad. Sci.*, 82, 821 (1959). [e] G. E. Arth, J. Fried, D. B. R. Johnson, D. R. Hoff, L. H. Sarett, R. H. Silber, H. C. Stoerk, and C. A. Winter, *J. Am. Chem. Soc.*, 80, 3161 (1958). [f] D. Taub, R. D. Hoffsommer, H. L. Slates, C. H. Kuo, and N. L. Wendler, *J. Am. Chem. Soc.*, 82, 4012 (1960). [g] J. S. Mills, A. Bowers, C. Djerassi, and H. J. Ringold, *J. Am. Chem. Soc.*, 82, 3399 (1960). [h] S. L. Steelman and E. R. Morgan, "Inflammation and Diseases of Connective Tissues", L. C. Mills and J. H. Meyer, Eds., W. B. Saunders, Philadelphia, 1961. [i] L. J. Lerner, A. R. Turkheimer, A. Bianchi, F. M. Singer, and A. Berman, *Proc. Soc. Exp. Biol. Med.*, 116, 385 (1964). [j] W. E. Dubin, *Proc. Soc. Exp. Biol. Med.*, 90, 115 (1955). [k] W. E. Dubin, B. J. Bowman, and R. O. Stafford, *Proc. Soc. Exp. Biol. Med.*, 94, 303 (1957). [l] J. A. Edwards, H. J. Ringold, and C. Djerassi, *J. Am. Chem. Soc.*, 82, 2318 (1960). [m] J. A. Edwards, H. J. Ringold, and C. Djerassi, *J. Am. Chem. Soc.*, 81, 3156 (1959). [n] A. Bowers, L. C. Ibanez, E. Denot, and R. Becerra, *J. Am. Chem. Soc.*, 82, 4001 (1960). [o] A. Bowers, E. Denot, M. Blanca, A. Sanchez, and H. J. Ringold, *Tetrahedron*, 7, 153 (1959). [p] R. I. Dorfmann, F. A. Kincl, and H. J. Ringold, *Endocrinology*, 68, 616 (1961). [q] Footnote omitted after revision. [r] S. L. Steelman, E. R. Morgan, and R. H. Silber, *Steroids*, 1, 163 (1963). [s] D. Branceni, G. Rousseau, and R. Jequier, *Steroids*, 6, 451 (1965). [t] A. E. Hydron, L. J. Lerner, and J. Achwartz, *Steroids*, 6, 247 (1965). [u] P. A. Desaulles and J. Samsel, *Hormones*, 2, 204 (1971). [v] H. G. von Schroder, M. Babej, and H. G. Vogel, *Arzneim.-Forsch.*, 24, 3 (1974). [w] Footnote omitted after revision. [x] J. Fried and A. Borman, *Vitam. Horm. (N.Y.)*, 16, 303 (1958). [y] R. Jequier, R. Plongeron, A. M. Verro-Orloff, and D. N. Branceni, *Therapie*, 20, 445 (1965). [z] J. Wieriks, W. Hespe, K. D. Jaitly, and B. L. van Kan, *Dermatologica*, 152, 181 (1976) (suppl 1). [aa] B. M. Phillips, F. J. Sanen, J. L. Leeling, T. L. Hammes, R. E. Hartnagel, L. F. Sancilio, O. J. Lorenzetti, and P. J. Kraus, *Toxicol. Appl. Pharmacol.*, 20, 522 (1971). [bb] G. Dipasquale, C. L. Rassaert, and E. McDongall, *Steroids*, 16, 663 (1970). [cc] H. J. Ringold, O. Mancara, C. Djerassi, A. Bowers, E. Batres, H. Martinez, E. Necocchea, J. Edwards, M. Velasco, C. Casa Campillo and R. I. Dorfmann, *J. Am. Chem. Soc.*, 80, 6464 (1958). [dd] W. H. Byrnes, L. E. Barnes, B. J. Bowman, W. E. Dubin, E. H. Morley, and R. O. Stafford, *Proc. Soc. Exp. Biol. Med.*, 91, 67 (1956).

by the blanching test, of 85 and 90, respectively, which is only slightly greater than the potency of the reference compound, hydrocortisone 17-butyrate (potency = 50).[24] The inclusion of the 17-hydroxy group into the betamethasone nucleus has the effect, as can be seen from Table IV, of substantially reducing potency. Thus, for the potencies of betamethasone 21-butyrate and betamethasone 21-isobutyrate to be correctly determined, excessively large positive values must be assigned to the 21-butyrate and 21-isobutyrate weight vectors.

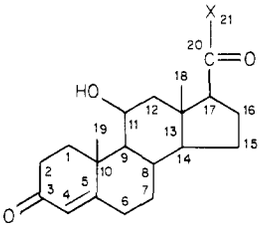
The results obtained for the granuloma study show some agreement with the vasoconstrictor study, since both predict that the 16,17-acetonide group and the 6-fluoro and 9-fluoro substituents will enhance potency, while saturation at the 1,2-position will reduce potency. In contrast to the vasoconstrictor study, however, the 17-hydroxy group is predicted to increase potency, while the 21-acetate group is predicted to decrease potency. The different contribution made by the 17-hydroxy group is exemplified by betamethasone and dexamethasone, which have different potencies measured by the two assays. One cannot, however, expect perfect correlation between the two biological tests, as the granuloma studies do not include contribution from the skin penetration–skin metabolism processes.

The linear pattern classifier obtained after training can be tested according to its ability to correctly classify the potency of unknown steroids not contained in the training set. This has been achieved for both studies by the so-called "leave one out" procedure.[25] In this procedure, a steroid was removed from the training set, and the remaining steroids were subjected to training in the usual manner. The steroid was then classified and returned to the training set, a second steroid was removed, and the training and classification procedure was repeated. This process was repeated for all members of the training set, whereupon the predictive ability of the linear pattern classifier could be determined. Although this procedure

(24) G. H. Phillips, "Mechanisms of Topical Corticosteroid Activity", L. Wildon and R. Marks, Eds., Churchill Livingstone, New York, 1976.

(25) B. R. Kowalski and C. F. Bender, *J. Am. Chem. Soc.*, 94, 5632 (1972).

Table III. Steroid Substituents Determined to be Significant[a] for the Pattern-Recognition Studies



human vasoconstrictor studies[b]			rat granuloma studies[b]		
no.	substituent	no.[c] occurrence	no.	substituent	no.[c] occurrence
1	saturated 1,2 bond	21	1	saturated 1,2 bond	40
2	6α-F	42	2	6α-F	21
3	9α-F	83	3	9α-F	32
4	16β-CH_3	40	4	6α-Cl	9
5	16α-CH_3	16	5	9α-Cl	4
6	17α-OH	37	6	11-oxo	16
7	17α-$OCOCH_3$	11	7	16α-OH	6
8	17α-$OCOC_2H_5$	20	8	16β-CH_3	9
9	17α-OCO-*n*-C_3H_7	12	9	16α-CH_3	17
10	17α-OCO-*n*-C_4H_9	14	10	17α-OH	58
11	21-OH	5	11	21-CH_2OH	40
12	21-OC_2H_5	3	12	21-CH_2OCOCH_3	38
13	21-OC_3H_7	4	13	16,17-acetonide	8
14	21-OCH_2SCH_3	3		6α-F + 9α-F	10
15	21-CH_2OH	38		saturated 1,2 bond + 17α-OH	29
16	21-CH_2OCOCH_3	17			
17	21-$CH_2OCOC_2H_5$	7			
18	21-CH_2OCO-*n*-C_3H_7	5			
19	21-CH_2OCO-*i*-C_3H_7	5			
20	21-CH_2Cl	10			
21	21-$COOCH_2Cl$	3			
22	16,17-acetonide	10			
	6α-F + 9α-F	34			
	6α-F + 21-CH_2OCOCH_3	9			
	9α-F + 16β-CH_3	37			

[a] Rejected substructural features are as follows. Vasoconstrictor study: 6α-CH_3; 11-oxo; 11β-Cl; 17α-$OCOCH(CH_3)_2$; 21-$CH_2OCOC(CH_3)_3$; 21-$CH_2OCOC_4H_9$; 21-CH_3; 21-OCH_2Cl; 21-$CH_2OPO(OH)_2$; 21-OCH_3; 17α-CH_3. Granuloma study: 6α-CH_3; 11β-Cl; 17α-$OCOC_3H_7$; 2α-CH_3. [b] See text. [c] Number of compounds included in the study and having the particular substituent.

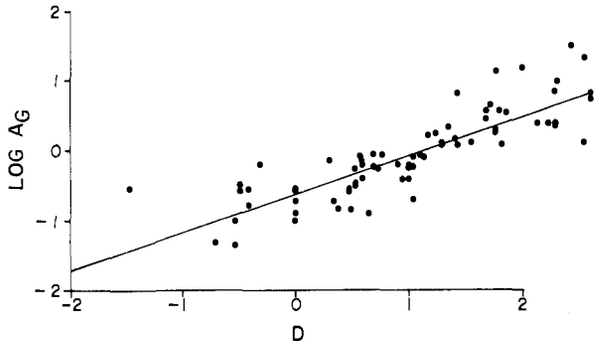


Figure 1. The plot of experimentally determined antigranuloma activities (log A_g) vs. calculated molecular ranking ($D = \sum w_i$) for the antiinflammatory steroids studied (see Table II) ($r = 0.853$).

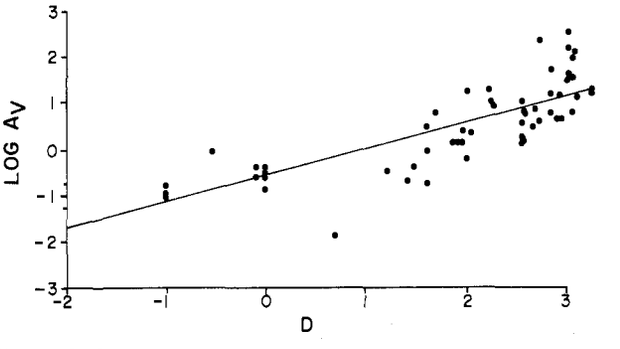


Figure 2. The plot of experimentally determined human vasoconstrictor activities (log A_v) vs. calculated molecular ranking ($D = \sum w_i$) for the antiinflammatory steroids studied (See Table I) ($r = 0.830$).

is computationally expensive, it does give a good indication of the performance of the linear pattern classifier. The predictive ability was fouind to be 87.7% for the vasoconstrictor study and 88.5% for the granuloma study. The following compounds were misclassified: in the vasoconstrictor studies, **15, 22, 29, 37, 38, 40, 41, 53, 56, 63, 78, 84,** and **113**; in the granuloma studies, **5, 18, 20, 26, 29, 32, 37, 39, 44** (the numbering refers to compounds in Tables I and II, respectively). Prediction is therefore good, since the probability of guessing the correct potency is, of course, 50% for a binary decision maker. An additional important test, as mentioned before, relates to the plots of the known biological activities against the calculated molecular ranking based on the contributions of the descriptor weight vectors. Figures 1 and 2 show the obtained plots based on the logarithm of the activity values.

The correlation, as expected, is better for the more quantitative rat granuloma test. The obtained plots were based on *all* compounds, including the ones that were misclassified in the "leave one out" procedure. A number of compounds have two rather different experimental values: The mean of these values was used in these cases. (If the misclassified values are left out, the correlation

Table IV. Sample Descriptor Values of the Various Substituents in the Steroids Studied

human vasoconstrictor data		rat granuloma data	
substituent	descriptor value	substituent	descriptor value
21-CH_2OCO-*i*-C_3H	1.11	16,17-acetonide	0.88
21-CH_2OCO-*n*-C_3H_7	1.10	6α-F	0.77
		9α-F	0.56
21-$COOCH_2Cl$	0.71	17α-OH	0.49
6α-F	0.68	16β-CH_3	0.47
21-CH_2Cl	0.54	9α-Cl	0.30
17α-$OCOC_2H_5$	0.51	6α-Cl	0.20
16,17-acetonide	0.50	16α-CH_3	0.16
		11-oxo	0.03
21-CH_2OCOCH_3	0.47	saturated 1,2 bond	−0.33
16α-CH_3	0.38	16α-OH	−0.40
9α-F	0.37	21-CH_2OH	−0.81
17α-$OCOC_3H_7$	0.22	21-CH_2OCOCH_3	−1.17
21-CH_2OH	0.17		
21-$CH_2OCOC_2H_5$	0.03		
17α-$OCOCH_3$	−0.06		
16β-CH_3	−0.07		
saturated 1,2 bond	−0.25		
17α-$OCOC_4H_9$	−0.38		
21-OCH_2SCH_3	−0.53		
21-OC_2H_5	−0.74		
21-OH	−1.12		
21-OC_3H_7	−1.26		
17α-OH	−1.32		

Table V. Sample Descriptor Values Obtained for Pairing Certain Substituents

vasoconstrictor data		granuloma data	
substituent	descriptor value	substituent	descriptor value
21-CH_2OCO-*i*-C_3H_7	1.00	16,17-acetonide	0.80
21-CH_2OCO-*n*-C_3H_7	0.82	6α-F	0.67
21-$COOCH_2Cl$	0.70	9α-F	0.50
17α-$OCOC_2H_5$	0.48	17α-OH	0.47
21-CH_2Cl	0.44	16β-CH_3	0.40
21-CH_2OCOCH_3	0.43	9α-Cl	0.39
16α-CH_3	0.41	6α-F + 9α-F	0.25[a]
6α-F + 9α-F	0.37[a]	6α-Cl	0.17
16,17-acetonide	0.35	16α-CH_3	0.16
6α-F	0.29	11-oxo	0.01
17-$OCOC_3H_7$	0.23	saturated 1,2 bond + 17α-OH	−0.02
21-CH_2OH	0.19		
16β-CH_3	0.15	saturated 1,2 bond	−0.29
6α-F + 21-CH_2OCOCH_3	0.11[a]	16α-OH	−0.34
		21-CH_2OH	−0.73
9α-F + 16β-CH_3	0.10[a]	21-CH_2OCOCH_3	−1.06
9α-F	0.02		
21-$CH_2OCOC_2H_5$	−0.04		
17α-$OCOCH_3$	−0.11		
saturated 1,2 bond	−0.27		
17α-$OCOC_4H_9$	−0.35		
21-OCH_2SCH_3	−0.40		
21-OC_2H_5	−0.66		
17α-OH	−0.98		
21-OH	−0.99		
21-OC_3H_7	−1.15		

[a] Synergistic increments.

coefficient improves to 0.872 in the case of the granuloma studies.)

This suggests that the derived pattern classifiers could be used to predict the antiinflammatory potency of untested, or unsynthesized, steroids. It is not suggested that the pattern-recognition technique should eliminate biological testing but rather that its role be complementary to that of testing. For example, in overtaxed testing programs it could establish a ranking order in which steroids were to be tested. A more important feature of the technique, however, is that by its analysis of previously tested steroids it allows the medicinal chemist to adopt a more rational to drug design. The results of the present study can strictly only be applied to those steroids containing the particular substituents considered in this paper. Steroids containing new substituents, or identical substituents in new positions, would have to be subjected to the pattern-recognition process. It is expected that the steroid data base will be *continuously extended* and improved as the pattern-recognition technique will be reapplied when the new experimental results become available.

Since all the structures studied contain more than one substituent, the linear learning machine program was extended to study the effect of combining certain pairs of substituents for both the vasoconstrictor and granuloma studies. The choice of which pairs of substituents to study was dictated by the chemical interest of the pairing and by the frequency with which that pairing occurred in the data.

The results obtained for both the vasoconstrictor and granuloma studies are shown in Table V. The results predict that a *synergistic* effect is in operation for certain pairings of substituents. For example, synergism is predicted between the 6- and 9-fluoro substituents, with the synergistic increment predicted to be 0.37; this is to say, the combined effect of a 6-fluoro and 9-fluoro pairing is 0.68, which is 0.37 more than the sum of the individual descriptors. A smaller synergistic effect is predicted to be in operation between the 6-fluoro and 21-acetate substituents and between the 9-fluoro and 16β-methyl substituents, the synergistic increment being 0.11 and 0.10, respectively.

In the granuloma study, synergism is also predicted between the 6-fluoro and 9-fluoro substituents, the synergistic increment being 0.25. The results predict an absence of synergism between the saturated 1,2 bond and the 17-hydroxy group.

It is difficult to rationalize the mechanism for synergism, although it is well known that small changes in the molecular structure of a steroid can have profound effects on biological activity. It may be that slight changes in conformation occur with the synergistic pair, which give the steroid a more favorable conformation for binding with the receptor. Another possible explanation may be the operation of long-range through-space interaction between the substituents of the synergistic pair. Whatever the mechanism of synergism, both assays seem to be sensitive to the effect. Nevertheless, our results predict that the potency of a steroid can be enhanced by the inclusion of certain pairs of substituents in the steroid nucleus, the most effect of those studied being the 6- and 9-fluoro compounds. It must be remembered, however, that our results ought not to be used to predict the overall clinical effectiveness of a steroid, since this study has not considered the occurrence of possible side effects. Synergism may play an important role not only in enhancing potency but also in decreasing unwanted side effects of a drug. If this proves to be the case, then the medicinal chemist will be in a position to synthesize a drug that most fully meets the clinical requirements or demands specified.

Conclusion

A pattern-recognition technique has been successfully applied to the elucidation of structure–activity relation-

ships in the antiinflammatory steroids. In addition to determining the contribution each individual substituent makes to potency, we predicted a synergistic effect to be in operation between certain pairs of substituents. These results allow the medicinal chemist to adopt a more rational approach to the design of potent antiinflammatory steroids.

Acknowledgment. Financial support by Otsuka Pharmaceutical Co. is gratefully acknowledged. A.J.H. is grateful for the financial support provided by the University of Florida.

Registry No. 1, 25122-46-7; 2, 50-02-2; 3, 5534-09-8; 4, 50-03-3; 5, 67-73-2; 6, 50-23-7; 7, 127-31-1; 8, 52-21-1; 9, 3093-35-4; 10, 25122-47-8; 11, 50-22-6; 12, 50-24-8; 13, 13609-67-1; 14, 356-12-7; 15, 37926-91-3; 16, 37926-78-6; 17, 913-42-8; 18, 59198-70-8; 19, 152-97-6; 20, 2002-29-1; 21, 312-93-6; 22, 2022-55-1; 23, 1524-88-5; 24, 76-25-5; 25, 5635-85-8; 26, 2152-44-5; 27, 987-24-6; 28, 378-44-9; 29, 53-36-1; 30, 84099-84-3; 31, 84099-85-4; 32, 4524-39-4; 33, 37926-75-3; 34, 37926-79-7; 35, 52619-01-9; 36, 52510-15-3; 37, 53-33-8; 38, 56933-60-9; 39, 84108-26-9; 40, 49697-38-3; 41, 37926-90-2; 42, 2240-28-0; 43, 5534-12-3; 44, 84099-86-5; 45, 29379-86-0; 46, 23640-97-3; 47, 23641-10-3; 48, 124-94-7; 49, 1177-87-3; 50, 37792-94-2; 51, 15180-00-4; 52, 37926-92-4; 53, 2693-06-3; 54, 4419-39-0; 55, 1597-82-6; 56, 23641-05-6; 57, 25122-48-9; 58, 302-25-0; 59, 37926-95-7; 60, 37927-21-2; 61, 37926-89-9; 62, 5534-14-5; 63, 75883-07-7; 64, 57524-89-7; 66, 638-94-8; 67, 514-36-3; 68, 83-43-2; 69, 84099-87-6; 70, 52510-28-8; 71, 25092-07-3; 72, 84099-88-7; 73, 37927-23-4; 74, 23641-03-4; 75, 37792-93-1; 76, 23674-85-3; 77, 806-29-1; 78, 37792-98-6; 79, 37926-93-5; 80, 382-67-2; 81, 641-77-0; 82, 5593-20-4; 83, 37926-96-8; 84, 4351-48-8; 85, 84099-89-8; 86, 84099-90-1; 87, 15845-96-2; 88, 84099-91-2; 89, 23640-96-2; 90, 42363-29-1; 91, 53-34-9; 92, 28971-62-2; 93, 2802-11-1; 94, 37926-76-4; 95, 84099-92-3; 96, 749-69-9; 97, 23674-86-4; 98, 25450-34-4; 99, 16463-74-4; 100, 2668-66-8; 101, 82034-75-1; 102, 5534-13-4; 103, 3863-59-0; 104, 84099-93-4; 105, 23641-07-8; 106, 84099-94-5; 107, 807-38-5; 108, 23641-01-2; 109, 630-44-4; 110, 25122-41-2; 111, 23641-09-0; 112, 23641-02-3; 113, 5534-18-9; 114, 23640-96-2; 116, 84099-95-6; 117, 72064-79-0; 118, 23641-08-9; 119, 37792-97-5; 120, 360-63-4; 121, 84099-96-7; 122, 25122-45-6.

Structure–Activity Studies of Configurationally Rigid Arylprostaglandins

Thomas K. Schaaf,*[†] M. Ross Johnson,*[†] Jay W. Constantine,[†] Jasjit S. Bindra,[†] Hans-Jürgen Hess,[†] and Walter Elger[‡]

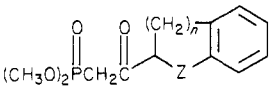
Central Research, Pfizer Inc., Groton, Connecticut 06340, and Research Laboratories, Schering A.G., Berlin/Bergkamen, D-1000 Berlin 65, Germany. *Received January 25, 1982*

Potent, albeit nonselective, smooth-muscle stimulant activity has been previously reported for 16-phenoxy- and 17-phenylprostaglandins, a finding that led to the design and development of the tissue-selective uterine stimulant sulprostone. As an extension of this work, analogues incorporating the 16-phenoxy and 17-phenyl substituents into the rigid indanyl, tetrahydronaphthyl, dihydrobenzofuryl, and dihydrobenzopyranyl ring systems were prepared and evaluated for uterine stimulant activity in vitro and diarrheal effects in vivo. Since these cyclic groups, with the exception of the indanyl, contain a chiral center, both optical antipodes were prepared. These studies demonstrate that ring size, heteroatom, and absolute configuration at C-16 are important determinants for potency and selectivity.

The lack of tissue selectivity and metabolic stability of the natural prostaglandins has prompted the synthesis of numerous analogues.[1] As part of a systematic investigation of structure–activity relationships, we previously reported that the 17-phenyl-ω-trinor- and 16-phenoxy-ω-tetranorprostaglandins exhibit potent, albeit relatively nonselective, smooth-muscle stimulant activity, a finding that was an important consideration in the design of the selective uterine stimulant sulprostone.[2] As an extension of this work, the flexible 17-phenyl and 16-phenoxy groups were incorporated into the rigid indanyl, tetrahydronaphthyl, dihydrobenzofuryl, and dihydrobenzopyranyl structures.[3] Since these cyclic substituents, with the exception of the indanyl, contain a chiral center, congeners containing each of the optical antipodes were prepared from resolved precursors. The synthesis and structure–activity relationships of these analogues are the subject of this paper.

Chemistry. The Corey synthesis,[4] which is ideally suited for the synthesis of PGE_2 and $PGF_{2\alpha}$ analogues modified in the *n*-amylcarbinol side chain, was used in the preparation of our analogues (Scheme I). Since the cyclic substituents were introduced by means of appropriately substituted, optically active phosphonates (Table I), it was necessary to resolve the acid precursors. Thus, sodium ethyl *tert*-butylmalonate[5] was condensed with xylylene dibromide to provide 2-(carboxyethyl)-2-carboxy-*tert*-butylindan, which was treated without purification with

Table I. Optical Rotation of Chiral Phosphonates



compd	Z	*n*	config	$[\alpha]_D$, deg (solv, *c*)
2a	CH_2	1		*a*
2b	CH_2	2	d[b]	+46.2 (MeOH, 1.00)
2c	CH_2	2	*l*[b]	−43.2 (MeOH, 1.00)
2d	O	1	d[b]	+32.4 (C_6H_6, 6.11)
2e	O	1	*l*[b]	−34.8 (C_6H_6, 6.11)
2f	O	2	*l*[b]	−57.3 ($CHCl_3$, 1.00)
2g	O	2	d[b]	+54.9 ($CHCl_3$, 1.17)

[a] Phosphonate is achiral. [b] Configuration of chiral carbon (pro C-16); designation refers to carboxylic acid precursor of phosphonates.

[†] Pfizer, Inc.
[‡] Schering A.G.

(1) A portion of this work has been previously presented: Johnson, M. R.; Schaaf, T. K.; Bindra, J. S.; Hess, H.-J. Abstracts: Fourth International Prostaglandin Conference, Washington, DC, May 17–31, 1979, p 56.
(2) Schaaf, T. K.; Bindra, J. S.; Eggler, J. F.; Plattner, J. J.; Nelson, A. J.; Johnson, M. R.; Elger, W.; Constantine, J. W.; Hess, H.-J. *J. Med. Chem.* **1981**, *24*, 1353, and references therein.
(3) For other configurationally restricted 17-phenyl congeners, see Fletcher, D. G.; Gibson, K. H.; Moss, H. R.; Sheldon, D. R.; Walker, E. R. H. *Prostaglandins* **1976**, *12*, 493.

0022-2623/83/1826-0328$01.50/0