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RECENT ADVANCES IN THE STRUCTURE–ACTIVITY RELATIONSHIPS OF SUBSTITUTED CORTICOSTEROIDS

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SUMMARY

The influence of the 6-azido-6-ene grouping on corticosteroid activity has been studied. The 6-azido-6-ene-corticosteroids were synthesized by reaction of the methanesulfonate esters of 6β,7β-dihydroxycorticosteroids with azide ion. These diols were the unexpected products of the reaction between OsO_4 and 6-ene-corticosteroids. Their β-stereochemistry was assigned unambiguously from their n.m.r. spectra and is contrary to that of some published assignments.

Anti-inflammatory activity was measured by the suppression of exudate in the rat granuloma pouch assay. Potency of 9α-unsubstituted corticosteroids was found to be increased by 5–8 times on introduction of the 6-azido-6-ene group. Introduction of this modification into 9α-fluorocorticosteroids enhanced potency in the absence of a 1,2-double bond and left the potency substantially unchanged in the presence of a 1,2-double bond.

INTRODUCTION

The search for cortisol analogues with modified glucocorticosteroid activity, particularly enhanced anti-inflammatory action, has been the source of much synthetic chemical effort over the last 25 years. This work has produced the 1,2-double bond, the 6α and 9α-F, the 6α,16α, and 16β-CH_3, and the 16α-OH as groups which, when introduced into the cortisol molecule either individually or in combinations have a beneficial effect upon the biological activity of corticosteroids. We would like to discuss here some of our findings obtained with a new activity modifying group that is clearly different from these, namely the 6-azido-6-ene group. The introduction of this function into a wide variety of natural and synthetic corticosteroids has produced some novel synthetic chemistry, as well as a number of biologically interesting corticosteroids. The first part of this paper will deal briefly with the synthetic methods developed, while in the second part the structure–activity relationships which have been derived will be discussed in greater detail.

Synthesis of 6-azido-6-ene-corticosteroids

The synthesis of 6-azido-6-ene-hydrocortisone 21-acetate and 6-azido-6-ene-cortisone 21-acetate has been reported from our laboratories [1] and is shown in Fig. 1. Acid catalyzed opening of the 6α,7α-epoxide (I) with azide ion gave 6β-azido-7α-hydroxycortisone 21-acetate (II). Acetylation at 7α followed by elimination of acetic acid with tetramethylammonium fluoride in CH_3CN furnished the desired 6-azido-6-ene-cortisone 21-acetate (III).

However, it was found that this sequence had serious limitations when applied to 9α-fluorocorticosteroids (Fig. 2). Epoxidation of the 9α-fluoro-4,6-diene-3-one (IV) with a wide variety of peroxy reagents gave poor yields of epoxide (Va). Also the rate of acid catalyzed opening of this 6α,7α-epoxide with azide ion was extremely slow. Interestingly though, the rate was found to be dependent on the substituent at 11, see Fig. 2. The adverse effect of the 9α-fluorine, presumably caused by conformational distortion of the epoxide or perhaps by the incipient 1-3 trans diaxial interaction between the 9α-fluorine and the 7α-oxygen substituents in the transition state (VIII), could be offset either by conversion of the 11β-hydroxy to the 11-ketone (Vc) or, to a lesser extent, to the 11β-acetoxy (Vb). Both of these modifications tend to move the 10 and 13-CH_3 groups further apart (Dreiding Models) and presumably a beneficial con-

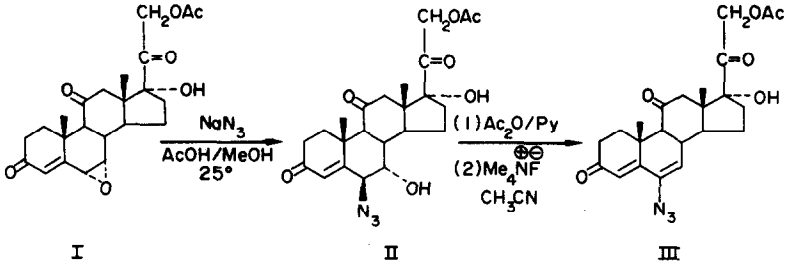

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Fig. 1.

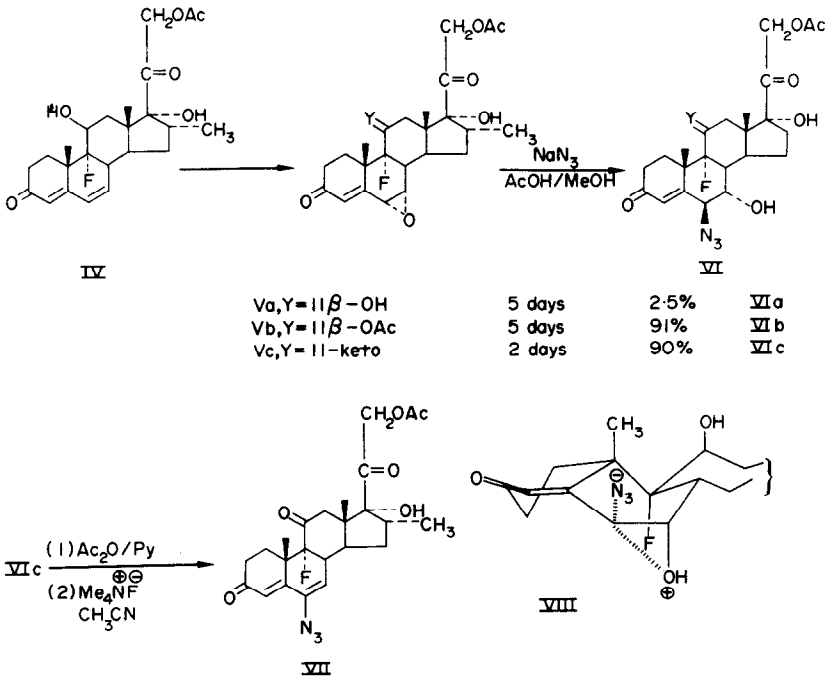


Fig. 2.

formational effect is transmitted through to the 6α,7α-epoxide.

These difficulties encouraged us to seek a new synthetic route to 6-azido-6-ene-steroids which would be of more general application. The literature contains many examples of the ready displacement of an allylic 6β-leaving group (*e.g.* IX; A = βBr, B = H: Fig. 3) by nucleophiles to give the 6α-substituted compounds. It was conjectured that, if in addition to a good leaving group at 6, there was a substituent at 7 which could be eliminated in a further reaction, then the 6-azido-6-ene function might be generated (Fig. 3). However, it was known [2] that a 6β,7α-trans arrangement of substituents (*e.g.* IX; A = βBr, B = αOAc) gives the 4-azido-4,6-diene-3-one as the major product. Therefore, the required system appeared to be a 6,7-*cis* arrangement of substituents. To test the proposed scheme we chose derivatives of *cis*-6,7-diols (*e.g.* IX; A = B = OMs) which should be readily available from the reaction of OsO_4 with 4,6-diene-3-ones. Our study of these compounds has fully justified our predictions in Fig. 3 and has led us to correct some published configurational assignments.

Cis-hydroxylation of three 6-ene-corticosteroids with OsO_4 was reported by Zderic, Carpio and Djerassi in 1959 [3] to give 6α,7α-diols, e.g. 6-ene-9α-fluorocortisol 21-acetate (XII) (Fig. 4) gave 6α,7α-dihydroxy-9α-fluorocortisol 21-acetate (XIV). The α-configuration was presumed "on the basis that attack from the rear is a more probable steric course considering the bulk of osmium tetroxide". We repeated this reaction and indeed found it to give one diol with the physical constants reported. However, the n.m.r. of this diol shows clearly that this assignment must be reversed; in fact, this reaction gives with complete stereospecificity, the 6β,7β-diol (XIII). Confirmation of these assignments came from examples where the reaction with OsO_4 gave the α-diol as well. Such was the case with 4,6-androstadiene-3,17-dione (XV) which gave the β-diol (XVI) in 80% and the α-diol (XVII) in 20% yields, respectively. The relevant n.m.r. signals are shown in Fig. 4, and a detailed analysis of these data will be reported in a future publication.

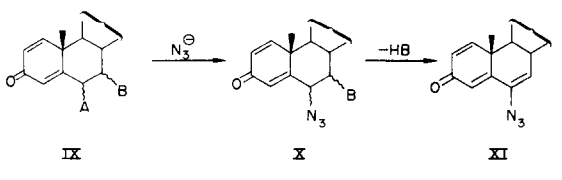
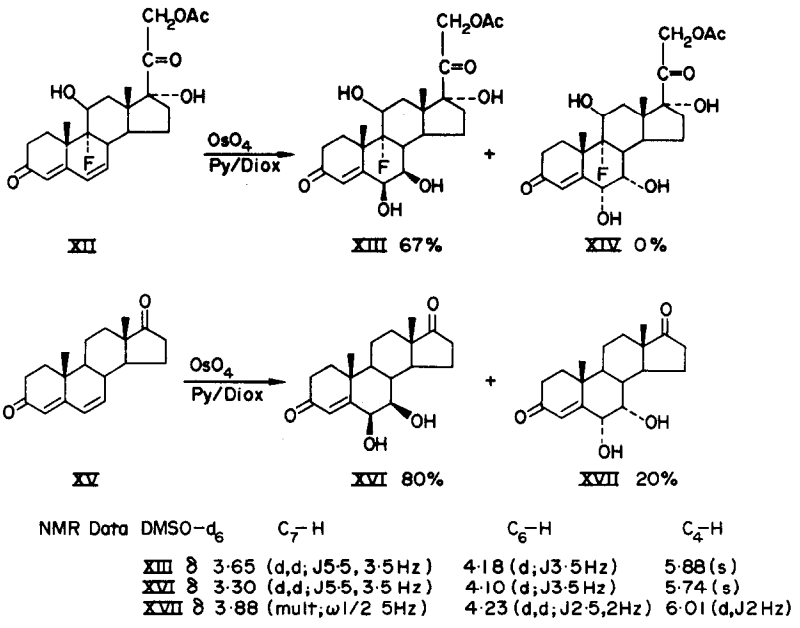


Fig. 3.

A similar n.m.r. analysis of the two diols from 6-ene-cortisone 21-acetate allowed the lower melting isomer to be assigned the 6β,7β-configuration. In this case the product of the reaction isolated by Djerassi *et al.*[3] was assigned correctly as the 6α,7α-diol but is in fact the minor product (α-diol 35%, β-diol 65%); its highly insoluble nature probably accounts for its isolation.

The *cis*-hydroxylation of 1,4,6-trien-3-ones with OsO_4 gave exclusively products of β-attack at the 6,7-double bond. For example, the reaction of 6-ene-betamethasone 21-acetate (XVIII) with OsO_4 was completely regiospecific and stereospecific giving only the 6β,7β-diol (XIX), Fig. 5. This extraordinary specificity was found to be the case with all 9α-halogenated-1,4,6-trien-3-ones but in the absence of a 9α-halogen attack also occurs at the 1,2-double bond. Thus, 6-ene-prednisolone 21-acetate (XX) gave the tetrol (XXII) in 20% yield, as well as the 6β,7β-diol (XXI), while the 11-keto analogue (XXIII) gave a 1ξ,2ξ-diol



NMR Data DMSO-d_6

	C_7–H	C_6–H	C_4–H
XIII	δ 3·65 (d,d; J5·5, 3·5 Hz)	4·18 (d; J3·5 Hz)	5·88 (s)
XVI	δ 3·30 (d,d; J5·5, 3·5 Hz)	4·10 (d; J3·5 Hz)	5·74 (s)
XVII	δ 3·88 (mult; ω1/2 5 Hz)	4·23 (d,d; J2·5, 2 Hz)	6·01 (d, J2 Hz)

Fig. 4.

(XXV) as the major product. However, in no case has any 6α,7α-diol been found and therefore the assignment for 6,7-dihydroxyprednisone 21-acetate (XXIV) made by Djerassi[3] must be reversed.

The reasons for such stereospecificity in the opposite direction to that which would be expected is not at all apparent from Dreiding models. However, it is more reasonable to use space-filling models to examine questions of steric hindrance and indeed CPK models of these 1,4,6-trien-3-ones show that the steroid skeleton is considerably bowed, Fig. 6. Thus, the α-face is concave and tends to be shielded while the β-face is convex and more exposed. In particular, the α-side of the 6,7-double bond is protected by the 14α-H, which is pushed underneath it by the bowing of the skeleton whereas on the β-side the 10-CH_3 and the 8β-H are pushed away from the double bond.

Two representative examples of the remainder of the synthetic sequence are shown in Fig. 7. Reaction of the 6β,7β-diols (XIX) and (XVI) with mesyl chloride in pyridine at 25°C for 2 h gave the 6β,7β-dimesylates (XXVII) and (XXXI) respectively in quantitative yield. Treatment of (XXVII) with NaN_3 in DMF for 24 h at 25°C gave the desired 6-azido-6-ene-betamethasone 21-acetate (XXIX, 45%) and the vinyl mesylate (XXX, 40%). Similarly, reaction of the 6β,7β-dimesylate (XXXI) under the same conditions gave 6-azido-4,6-androstadiene-3,17-dione (XXXII, 38%), identical with the compound synthesized by the 6α,7α-epoxide route (Fig. 1), and the vinyl mesylate (XXXIII, 12%).

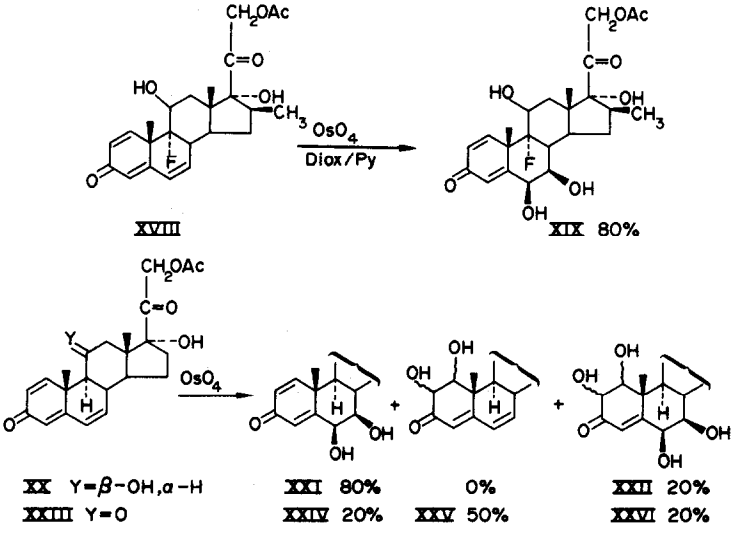


Fig. 5.

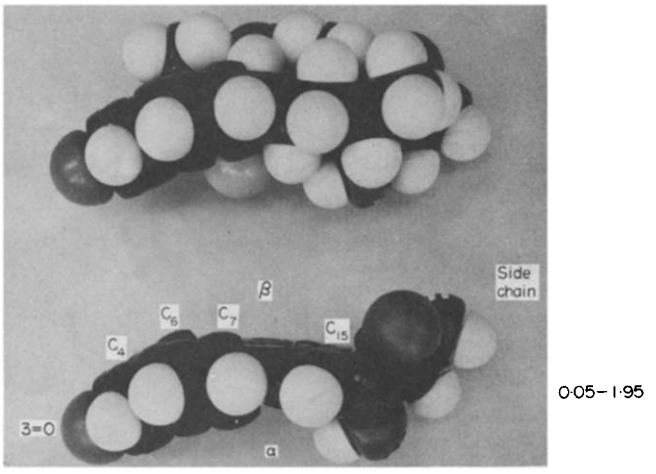


Fig. 6.

However, reaction of the isomeric 6α,7α-dimesylate (XXXIV) with azide ion under a variety of conditions gave only the vinyl mesylate (XXXIII).

We envisage the reaction to proceed by an SN2 displacement at the 6-position (the most reactive allylic mesylate) by azide ion to give the 6α-azido-7β-mesyloxy compound (*e.g.* XXVIII) which, under the basic conditions of the reaction readily loses methanesulfonic acid (6β-H and 7β-OMs) to introduce the 6,7-double bond. This competes with the less ready, direct trans diequatorial elimination of the 6α-H and the 7β-OMs to give the vinyl mesylate (*e.g.* XXX). However, in the case of the 6α,7α-dimesylate (XXXIV), the trans diaxial loss of methanesulfonic acid (6β-H and 7α-OMs) is highly favored and the vinyl mesylate becomes the sole product of the reaction.

RESULTS AND DISCUSSION

Structure–activity relationships of 6-azido-6-ene-corticosteroids

The primary assay used to measure antiinflammatory potency was the rat granuloma pouch technique of Robert and Nezamis[4]. Female rats (Charles River), weighing approximately 140 g were used throughout. Compounds, as crystalline suspensions in 0·2 ml of aqueous carboxymethylcellulose vehicle, were administered subcutaneously 1 ×/day/4 days starting at the time of pouch formation. The rats were sacrificed 24 hours after the last injection and the exudate volume was compared with that from rats treated with the standard prednisolone acetate. This value, which is a measure of anti-inflammatory activity, is recorded for the compounds under study in Table 1.

The data show that introduction of the 6-azido-6-ene-grouping into 9α-unsubstituted corticosteroids increases anti-inflammatory potency by 5–8 times. When this modification is introduced into 9α-fluoro corticosteroids anti-inflammatory potency is enhanced in the absence of a 1,2-double bond and is substantially unchanged when a 1,2-double bond is present.

Introduction of the 6-azido-6-ene group into cortisol 21-acetate and cortisone 21-acetate had a profound effect upon anti-inflammatory potency, Table 1. Thus 6-azido-6-ene-cortisol 21-acetate (3) and 6-azido-

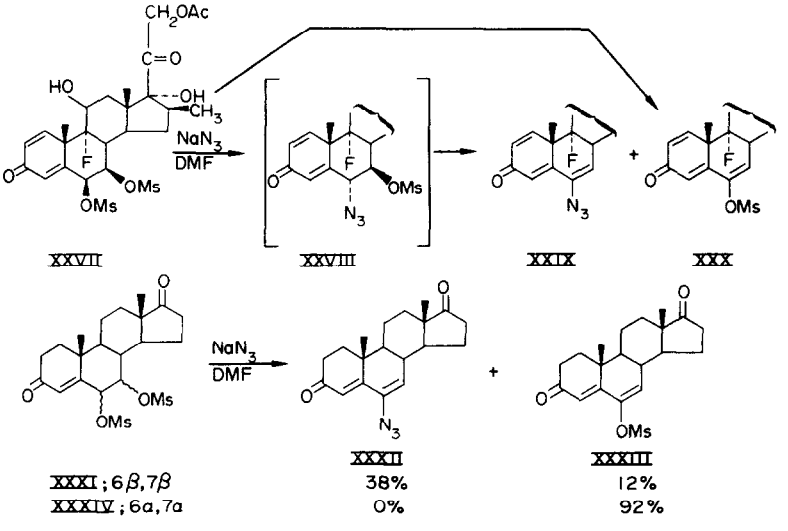


Fig. 7.

Table 1. Antiinflammatory potencies of 6-azido-6-ene-corticosteroids

Compound number	Compound name	Exudate suppression (Potency × prednisolone 21-acetate)
1	Cortisol 21-acetate	0·2
2	6-Ene-cortisol 21-acetate	⩽0·2
3	6-Azido-6-ene-cortisol 21-acetate	1·2
4	6-Azido-6-ene-cortisone 21-acetate	1·6
5	Prednisolone 21-acetate	1
6	Prednisolone	0·8
7	6-Ene-prednisolone	<0·2
8	6-Azido-6-ene-prednisolone	4·2
9	16α-Methyl-9α-fluorocortisol 21-acetate	2·0
10	6-Ene-16α-methyl-9α-fluorocortisol 21-acetate	3·5
11	6-Azido-6-ene-16α-methyl-9α-fluorocortisol 21-acetate	10
12	16β-Methyl-9α-fluorocortisol 21-acetate	1·7
13	6-Ene-16β-methyl-9α-fluorocortisol	2·2
14	6-Azido-6-ene-16β-methyl-9α-fluorocortisol	3·5
15	6-Ene-16α-methyl-9α-fluorocortisone 21-acetate	6·7
16	6-Azido-6-ene-16α-methyl-9α-fluorocortisone 21-acetate	13·3
17	Dexamethasone	35
18	6-Ene-dexamethasone	21
19	6-Azido-6-ene-dexamethasone	31·5
20	11-Dehydrodexamethasone	33·5
21	6-Ene-11-dehydrodexamethasone	5·7
22	6-Azido-6-ene-11-dehydrodexamethasone	11·5
23	Betamethasone	12
24	6-Ene-betamethasone	3
25	6-Azido-6-ene-betamethasone	10·5
26	11-Dehydrobetamethasone	12·3
27	6-Ene-11-dehydrobetamethasone	1·6
28	6-Azido-6-ene-11-dehydrobetamethasone	9·6
29	6-Ene-1,2-dihydrotriamcinolone acetonide 21-acetate	2·5
30	6-Azido-6-ene-1,2-dihydrotriamcinolone acetonide 21-acetate	4·5
31	Triamcinolone acetonide	14·6
32	Triamcinolone acetonide 21-acetate	6
33	6-Ene-triamcinolone acetonide 21-acetate	6
34	6-Azido-6-ene-triamcinolone acetonide 21-acetate	6
35	Triamcinolone 16α,21-diacetate	1·3
36	6-Ene-triamcinolone 16α,21-diacetate	0·4
37	6-Azido-6-ene-triamcinolone 16α,21-diacetate	1·9
38	21-Desoxydexamethasone	3·8
39	6-Ene-21-desoxydexamethasone	2·3
40	6-Azido-6-ene-21-desoxydexamethasone	6·3

6-ene-cortisone 21-acetate (4) were 6–8 times more potent than cortisol 21-acetate (1) and indeed were even more potent than prednisolone 21-acetate (5). This enhancement appears even more dramatic with the comparison of this activity with the 6-unsubstituted compound, 6-ene-cortisol 21-acetate (2), which was essentially inactive in this assay. Similarly, introduction of the 6-azido-6-ene grouping into prednisolone effected an increase in potency to 5 times prednisolone (6). Here also the sharp drop in potency found with the introduction of the 6,7-double bond was more than overcome by the further introduction of the 6-azide group [(6)→(7)→(8)].

A similar pattern emerged from the 16-methyl-9α-fluorocortisol series where the effect of the 6-azido-6-ene modification was to increase potency by 5 times for the 16α-methyl compound (11) and by 2 times for the 16β-methyl analogue (14). Thus 6-azido-6-ene-16α-methyl-9α-fluoro-cortisol 21 acetate (11) is 10 times and 6-azido-6-ene-16β-methyl-9α-fluoro-cortisol (14) is 3·5 times more potent than prednisolone 21-acetate. It should be pointed out that the lower values for 16β-methyl corticosteroids are a consistent feature of this assay in our laboratories. Also, in the 16α-methyl series, there was little difference in potency between the 11-keto and the 11β-hydroxy-6-azido-6-ene compounds [Table 1, (11) and (16)]. However, these two series of compounds did differ from the cortisol and prednisolone cases in that introduction of the 6,7-double bond did not decrease potency; the variable nature of the 6,7-double bond on anti-inflammatory potency has been previously noted [5].

However, introduction of 6-azido-6-ene grouping into the highly potent corticosteroids, dexamethasone

(17) and betamethasone (23), had very little effect on anti-inflammatory potency. In these compounds the 6,7-double bond caused a large reduction in potency, (18) and (24), which was only barely compensated for by the further introduction of the azide group. Thus 6-azido-6-ene-dexamethasone (19) and 6-azido-6-ene-betamethasone (25), although being the most potent azidocorticosteroids so far, are only about as potent as their parents, dexamethasone and betamethasone respectively. The deactivating effect of the 6,7-double bond was even more pronounced with 6-ene-11-dehydrodexamethasone (21) and 6-ene-11-dehydrobetamethasone (27) which were between 1/6 and 1/8 as potent as their parent compounds, (20) and (26) respectively. Substitution by the azide group then gave 6-azido-6-ene-11-dehydrodexamethasone (22), which was substantially less potent than its parent (20), and 6-azido-6-ene-11-dehydrobetamethasone (28) which was only slightly less potent than 11-dehydrobetamethasone (26).

If only the effect of introduction of the 6-azide group into the 6-ene-corticosteroid is considered then all the fluorinated corticosteroids present a consistent picture; *viz* a potency increase of 2 to 6 times. However the *net* effect of the 6-azido-6-ene modification is to increase potency in the absence of a 1,2-double bond and to leave the potency substantially unchanged when a 1,2-double bond is present.

This pattern is maintained in two other fluorinated corticosteroid series, the triamcinolone 16α,21-diacetate and the triamcinolone 16α,17α-acetonide series, both of which contain a 16α-oxygen function instead of a 16-methyl. Thus 6-azido-6-ene-triamcinolone acetonide 21 acetate (34) is equipotent to its parent, triamcinolone acetonide 21-acetate (32) whereas the 1,2-dihydro-6-azido-6-ene compound (30) is twice as potent as the 1,2-dihydro-6-ene compound (29). Also, 6-azido-6-ene-triamcinolone 16α,21-diacetate (37) is equipotent to triamcinolone 16α,21-diacetate (35). An apparent exception appears to be 6-azido-6-ene-21-desoxydexamethasone (40). This compound, although considerably less potent than 6-azide-6-ene-dexamethasone (19), is twice as potent as its parent, 21-desoxydexamethasone (38).

The two most active compounds, 6-azido-6-ene-dexamethasone (19) and 6-azido-6-ene-betamethasone (25), have been further tested in a number of other assays, see Table 2. The corticosteroid activity of both compounds carried over into two other species, the mouse and the dog, although relative potencies have not been obtained. In the rat electrolyte assay neither compound showed any salt or water retention while in the adjuvant arthritic rat the effects on paw volumes, erythrocyte sedimentation rate, and spleen weights closely paralleled the effects of their respective parent compounds. However, in the rat ulcer assay both azidocorticosteroids produced significantly fewer ulcers than did dexamethasone. A possible effect on blood pressure due to the azide group was negative at 120 μg/kg in the cat. This dose supplied 12 times the amount of azide that produced a significant drop in blood pressure when supplied as azide ion.

In Table 3 a comparison is made between the effects of the 6-azido-6-ene modification and of other 6-substituents on anti-inflammatory potency. For the non-fluorinated corticosteroids, cortisol and prednisolone, the effect of the 6-azido-6-ene grouping is intermediate between that of the 6α-CH_3 and the 6α-F whereas in the three fluorinated examples it falls below that of both. The variability of the effect of the 6-azido-6-ene modification is similar to the variation shown by the 6α-F but different from that of the 6α-CH_3 which has an essentially constant effect on potency. In the dexamethasone and 1,2-dihydrodexamethasone series the 6-azido-6-ene grouping has a remarkably similar effect on potency to that of the 6-CH_3-6-ene group; it is interesting to speculate on what the —CH_3 and the —N=$N^{\oplus}$=$N^{\ominus}$ groups could have in common!

To enable us to look more closely at the relationship between molecular conformation and biological activity of corticosteroids, the 3-dimensional structure of 6-azido-6-ene-betamethasone 21-acetate has been determined by X-ray crystallographic analysis. We

Table 2. Other biological activities of 6-azido-6-ene-corticosteroids

Species	Assay	Route of administration	6-Azido-6-ene-dexamethasone	6-Azido-6-ene-betamethasone
Rat	Electrolytes[a]	SC	No Na, K or water retention	
Rat	Adjuvant arthritis[b]	SC	≃ Dexamethasone	≃ Betamethasone
Rat	Ulcerogenicity[c]	SC	< Dexamethasone	< Dexamethasone
Mouse	Thymus/carrageenan paw[d]	P.O.	Active	Active
Dog	Eosinopenia[e]	P.O.	Active	Active
Cat	Autonomic challenge	I.V.	Inactive at 120 μg/kg	

[a] Assayed by the Mason Research Institute, Worcester, Mass.

[b] The method of Pearson and Wood [Pearson C. and Wood F.: *Am. J. Path.* **42** (1963) 73–96] was modified in that steroid treatment was initiated 14 days after administration of the adjuvant. The animals were sacrificed 7 days later and paw volumes, erythrocyte sedimentation rates, and spleen weights were measured.

[c] Robert A. and Nezamis J. E.: *Archs Path.* **77** (1964) 407–423.

[d] Mice (Carworth Farms CF1) weighing 18–22 gm were challenged 1 h after oral steroid administration by carrageenan (injection of 0·1 ml of a 1% solution into the plantar surface of one hind paw). After 3 h the animals were sacrificed and the thymus and hind paw weights were measured.

[e] Collins E. J., Aschenbrenner J. and Nakama M.: *Steroids* **20** (1972) 543–554.

Table 3. Effect of 6-substituents on anti-inflammatory potency

Parent corticosteroid (Potency = 1)	Anti-inflammatory potency relative to parent corticosteroid[a] 6-substituent			
	6α-CH_3[b]	6-CH_3-6-ene[c]	6-N_3-6-ene	6α-F[b]
Cortisol	3		6–8	9
Prednisolone	2		5	6–8
Dexamethasone	2·7	0·75	0·92	2–2·5
		0·32 (1,2-dihydro)	0·3 (1,2-dihydro)	
Triamcinolone 16α,21-diacetate	2		1	
Triamcinolone 16α,17α-acetonide 21-acetate	2[d]		1	

[a] Rat systemic granuloma data except where noted. Each figure, when calculated from literature figures, refers only to cases where values for both modified and parent compounds are available from the same authors in the same publication.

[b] Data taken from Sarett L. H., Patchett A. A. and Steelman S. L.: In *Progress in Drug Research* (Edited by E. Jucker). Birkhauser Verlag, Basel, Vol. 5, p. 11, pp. 11–153.

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[d] Rat thymolysis; data taken from Ringler I.: In *Methods in Hormone Research* (Edited by R. I. Dorfman). Academic Press, New York and London, Vol. III, p. 227, pp. 234–292.

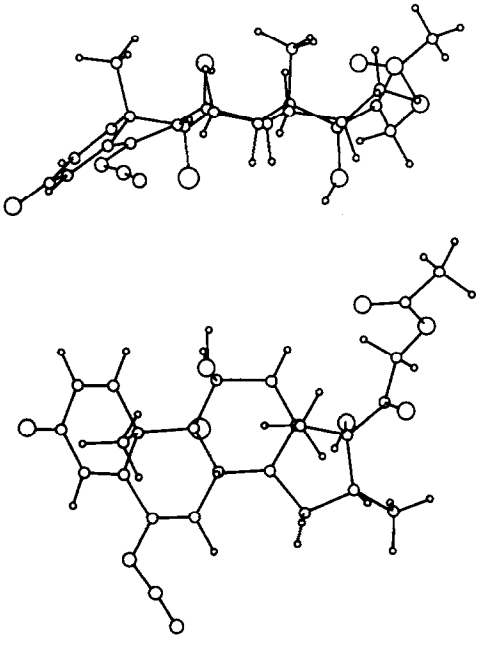

Fig. 8.

would like to thank Dr. Olga Kennard, Dr. L. R. Nassimbeni and Dr. G. M. Sheldrick of the University of Cambridge, England for this determination [6] and for the two perspective views of the molecule in Fig. 8. As preliminary comments we would like to point out that the marked bowing of the skeleton mentioned earlier in this paper in connection with 6-ene-corticosteroids is clearly evident here and also that the azide group lies almost in the plane of the unsaturated system of Rings A and B.

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