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NEW BIOLOGICALLY ACTIVE PREGNAN-21-OIC ACID ESTERS

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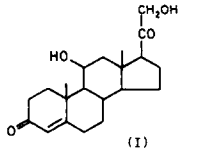
SUMMARY

Among the metabolites of fluocortolone (IV), isolated from human urine, there was found fluocortolone-21-acid (XI). This metabolite and several ester derivatives were synthesized by different routes starting from fluocortolone. The synthetic methods used to prepare the fluocortolone acid esters were extended to other corticoid derivatives. Several pregnan-21-oic acid esters possess the unique activity that they are topical anti-inflammatory agents with no side effects. Even when administered at high dosages, the usual systemic activity associated with corticosteroid treatment could not be observed.

Until 1964, the therapeutically useful corticoids with pronounced anti-inflammatory activity were all derived from either cortisol or 21-deoxy-cortisol. Derivatives such as corticosterone (I) [1, 2] or 1-dehydrocorticosterone (II) [3] in which the 17-hydroxy group is absent, are either totally devoid of anti-inflammatory activity or are effective only to a very slight degree. However, as shown by Raspé *et al.*[4], the activity of 1-dehydrocorticosterone (II) could be increased considerably by the introduction of a 16α-methyl group. A substitution of this sort is also responsible for diminishing sodium retention properties and other side effects asociated with corticoid therapy. Introduction of 6α-fluorine into the 16α-methyl derivative (III) yields the potent corticosteroid fluocortolone (IV) [5].

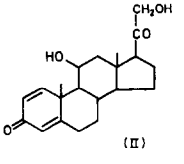
Gerhards *et al.*[6] have carried out a detailed study of the metabolism of fluocortolone (IV) in humans and have isolated a number of derivatives from the urine of patients administered fluocortolone orally. Among the directly extractable compounds were found 6β-hydroxylated metabolites (V–VIII), from which the two 11-hydroxy derivatives (V) and (VI) could be identified as the major metabolites. From the glucuronoside fraction there was obtained unchanged fluocortolone (IV), its corresponding 11-ketone (IX) and a pentahydroxy derivative (X) of undetermined structure. In the sulfate fraction, there could be demonstrated that, in addition to fluocortolone (IV), a water soluble carboxylic acid was present which was identified as the 21-acid of fluocortolone (XI).

No anti-inflammatory activity



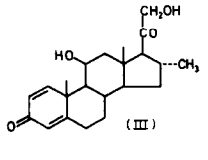
(I)

Slightly active



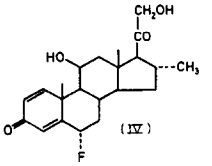
(II)

Increasing activity



(III)

Strong activity



(IV)

fluocortolone

As this C-21-acid represents a new corticoid structure type, we considered it to be an interesting problem to prepare this metabolite and a number of ester derivatives (XII) synthetically to test these esters for anti-inflammatory activity.

Animal studies and clinical findings have demonstrated that a free hydroxyl group at C-21 is not essential for the topical anti-inflammatory activity of corticoid preparations. For example, it is only necessary to consider the effectiveness of corticoid-21-acylate and -sulfate derivatives as well as the strong anti-inflammatory activity of those compounds in which the 21-hydroxy group is replaced by a fluorine, chlorine or hydrogen atom.

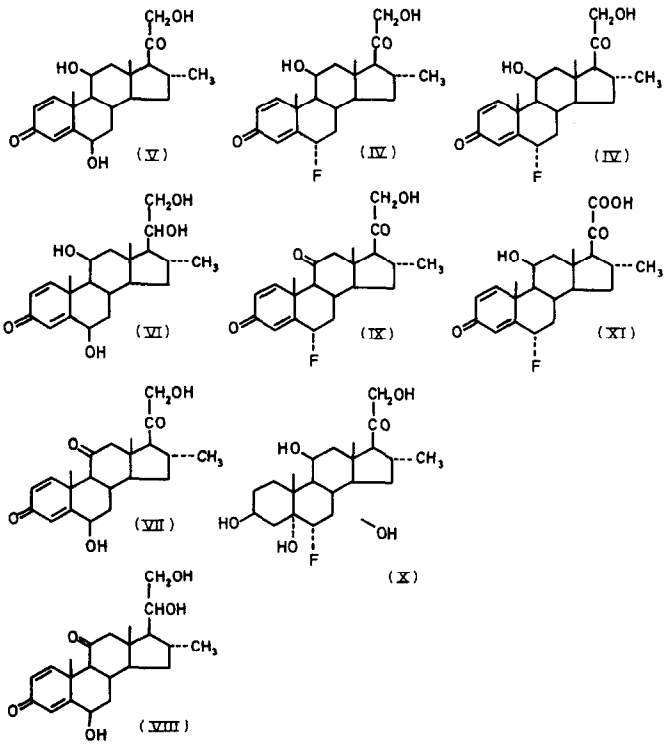
We considered it possible that these fluocortolone-21-acid esters (XII) would possess normal corticosteroid activity *per se* and assumed further that these derivatives, after resorption, would be hydrolyzed to the free acid (XI). This acid, however, should not exhibit significant corticoid activity as it would be rapidly eliminated from the body as a natural metabolite of fluocortolone.

The goal of our synthetic efforts was, therefore, the preparation of a topically active corticoid devoid of the usual systemic effects.

The major difficulty associated with the transformation of fluocortolone (IV) to the α-keto-acid (XI) was considered to be the selective oxidation of the side chain without attack on the 11β-hydroxyl group. A similar conversion however, involving the oxidation of the corticosterone side chain to the α-ketoester has been previously described by Lewbart and Mattox [7] in 1963. Methanolic cupric acetate is capable of oxidizing the 21-alcohol of the α-ketol (XIII) to the corresponding aldehyde (XIV) within

Metabolites of fluocortolone

free extractable glucuronoside fraction sulfate fraction

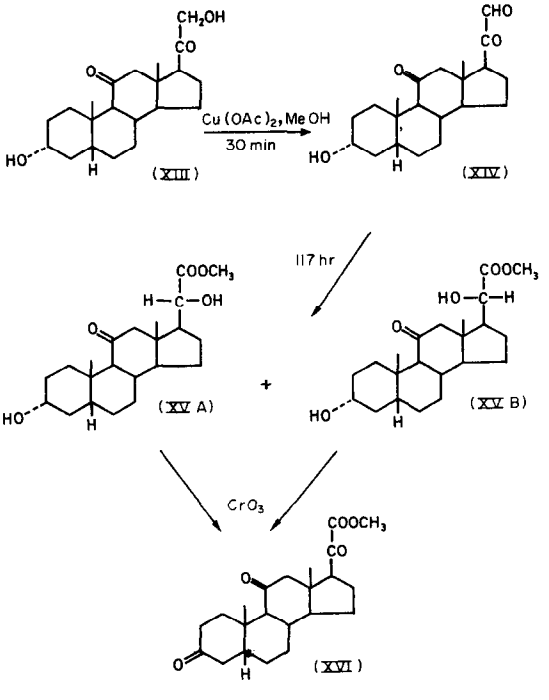


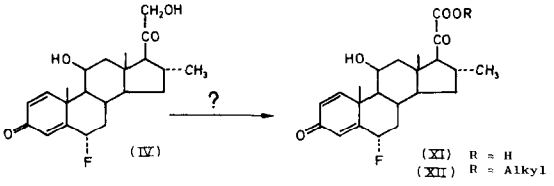
E.Gerhards, B.Nieuweboer, G.Schulz and H.Gibian, Acta Endocrin. 68,98(1971)

30 min. After much longer reaction times, the initially formed α-ketoaldehyde (XIV) rearranges to an epimeric mixture of 20-hydroxy-21-methyl esters (XV). The mixture of alcohols was separated chromatographically and each epimer was oxidized with chromic acid to the corresponding α-ketoester (XVI).

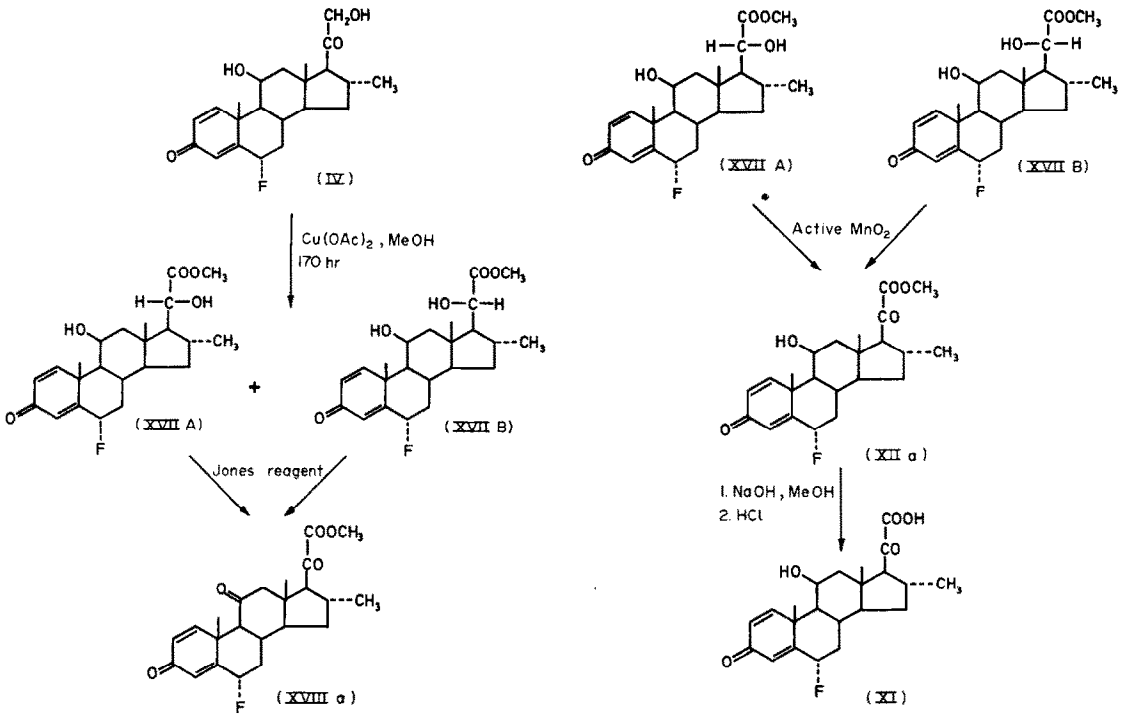
We have applied this procedure to fluocortolone and have also isolated a mixture of hydroxy esters (XVII) after correspondingly long reaction times by oxidation with cupric acetate. The C-20-epimers were separated and each was converted into the triketo ester (XVIIIa) by treatment with Jones reagent.

We were then interested in finding a reagent capable of selectively oxidizing the hydroxyesters (XVII) to the α-ketoester (XIIa). After some experimentation, it became evident that active manganese dioxide was the most suitable reagent which could be utilized for this purpose.





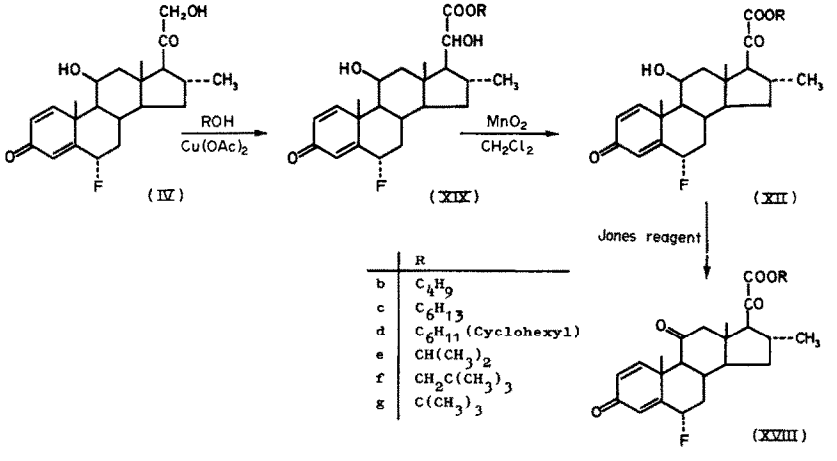
M.L.Lavbart and V.R.Mattox, J.Org.Chem. 28,1779(1963)



Following this procedure, it was possible to prepare the desired fluocortolone acid-methyl ester (XIIa) in good yields from the epimeric hydroxy-esters (XVII). Saponification of the ester (XIIa) with methanolic sodium hydroxide followed by treatment with hydrochloric acid yielded the free acid (XI) which was identical to the metabolite isolated from natural sources. This derivative is a relatively strong organic acid and is stable for extended periods of time when stored at room temperature. However, at higher temperatures the acid is found to decompose.

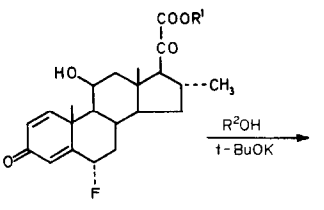
In order to prepare other ester derivatives in this series, we investigated the reaction of fluocortolone (IV) with cupric acetate in different alcohols to give the corresponding 20-hydroxy-esters (XIX) followed by oxidation with Braunstein reagent. It was found, that the formation of the 20-hydroxy-esters with butanol, hexanol, cyclohexanol, isopropyl alcohol, neopentyl alcohol and *tert*-butyl alcohol proceeded similarly as with methanol. The only difference was that the sterically hindered alcohols required somewhat longer reaction time. From the mixture of C-20 epimeric hydroxy esters (XIX) there could be obtained in each instance the pure 20α- and 20β-hydroxy esters by chromatography. The chromatographic fractions containing a mixture of C-20 epimeric alcohols were, in most instances, converted directly to the corresponding fluocortolone acid-ester (XII) by treatment with manganese dioxide. The 11-ketones (XVIII) were sometimes produced in small quantities by oxidation with active manganese dioxide for prolonged periods of time. When desired, however, the 11-ketones could be obtained in good yields by oxidation of the fluocortolone acid-esters (XII) with Jones reagent.

Additional esters of fluocortolone acid were prepared by means of a mild alkaline transesterification



procedure utilizing, as catalyst, either sodium ethylate, potassium *tert*-butylate or aluminium isopropylate. From either the methyl or butyl ester of fluocortolone acid, there could be prepared, following this method, the ethyl, propyl, pentyl, allyl and propargyl esters, to name only a few. The transformation proceeds smoothly by either overnight reaction at room temperature or at higher temperatures. In order to form the esters of secondary alcohols, such as 2-butanol, 2-octanol or menthol there were required longer reaction times and higher temperatures. The formation of the *tert*-butyl ester (XIIg) could be achieved with difficulty under these harsher reaction conditions.

At this point when the synthesis of such derivatives no longer posed a problem, a number of test results were on hand which demonstrated that these esters of fluocortolone acid and the corresponding 11-ketones possess remarkable pharmacological activities. In the vasoconstriction test carried out on human skin, it was found that an entire series of these esters were locally active anti-inflammatory agents. In contrast, however, by subcutanous or oral administration in the adjuvans paw edema test, spleen and thymus weight test and eosinophile test the new preparations were totally inactive. Furthermore, they do not influence the glyconeogenesis in the liver and have no affect on the sodium–potassium balance. The usual systemic activity observed by local application of conventional corticoid preparations are not to be feared with these new derivatives. On the basis of these findings, the fluocortolone acid-butyl ester (XIIb) has been suggested for extensive dermatological testing in clinical trials.



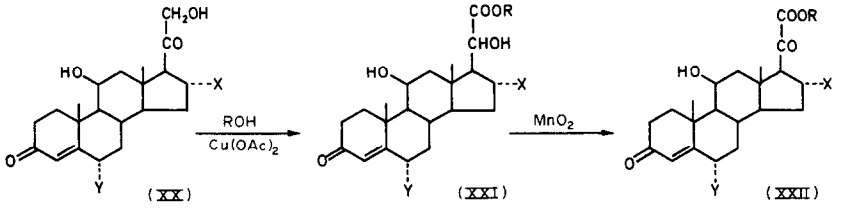
(XII) (XII)

	R^1
a	CH_3
b	C_4H_9

	R^2
h	C_2H_5
i	C_3H_7
k	C_5H_{11}
l	$CH_2-CH=CH_2$
m	$CH_2-C\equiv CH$
n	$CH(CH_3)C_2H_5$
o	$CH(CH_3)C_6H_{13}$
p	menthyl
g	$C(CH_3)_3$

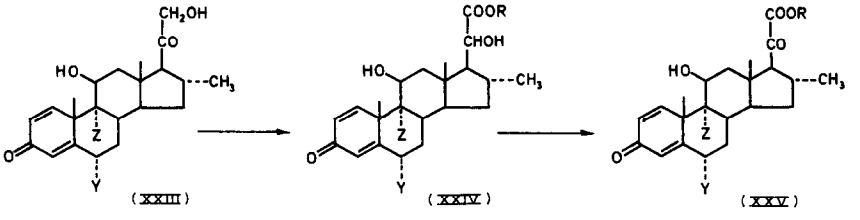
These pharmacological results gave us cause to prepare further derivatives of α-hydroxy-carboxylic acids in the corticosterone, 16α-methylcorticosterone and 6α-fluoro-16α-methylcorticosterone series (general formula XX) which were further converted to the α-ketoesters (XXII) by means of the oxidation with active manganese dioxide.

Also, in the 1-dehydrocorticosterone series (XXIII), the 16α-methyl derivative as well as desoxymethasone, diflucortolone and clocortolone were subjected to the same transformation to yield the α-ketoesters (XXV).



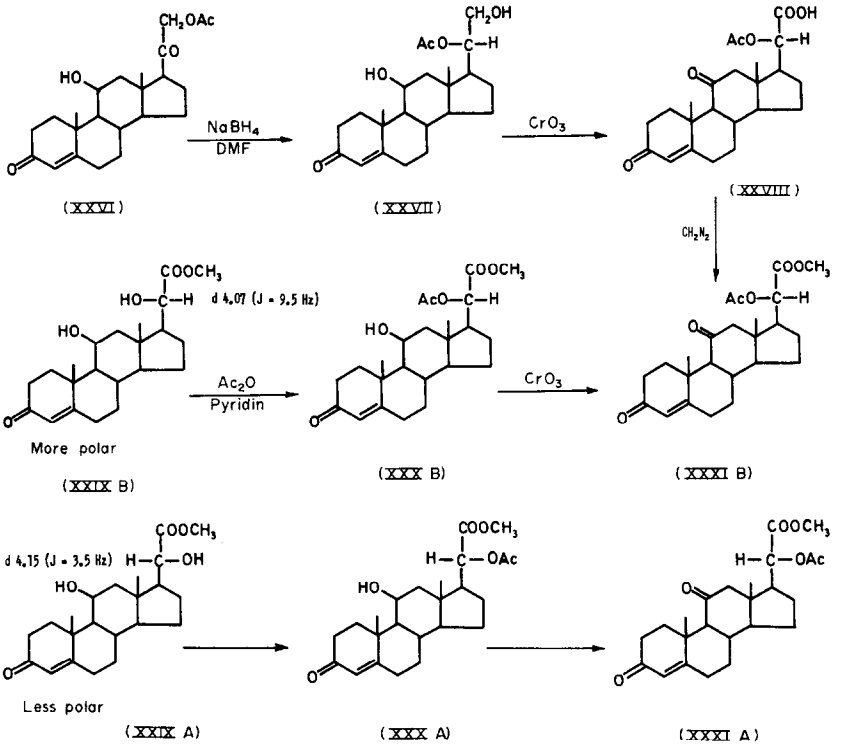
(XX) (XXI) (XXII)

starting material	X	Y	R
corticosterone	H	H	CH_3, C_4H_9
16α-methylcorticosterone	CH_3	H	C_4H_9
6α-fluoro-16α-methylcorticosterone	CH_3	F	C_4H_9



(XXIII) (XXIV) (XXV)

starting material	Y	Z	R
16α-methyl-1-dehydrocorticosterone	H	H	C_4H_9
desoxymethasone	H	F	CH_3
diflucortolone	F	F	CH_3, C_2H_5, C_4H_9
clocortolone	F	Cl	CH_3, C_4H_9, $CH(CH_3)_2$

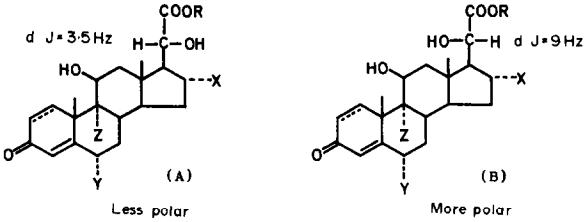


As was mentioned previously, from the epimeric mixtures of 20-hydroxyesters (XXI) and (XIV) it was possible to obtain the pure 20α- and 20β-epimers by chromatography.

The stereochemistry of the alcohols at C-20 could be assigned by correlation with a corticosterone derivative of known configuration. The reduction of corticosterone acetate (XXVI) with sodium borohydride in dimethylformamide following a literature procedure [8, 9] yields the 20β-acetate (XXVII) which was oxidized with Jones reagent and subsequently reacted with diazomethane to yield the acetoxy ester (XXXIB) of known configuration.

The two hydroxyesters (XXIXA) and (XXIXB) obtained by oxidation of corticosterone with cupric acetate were acetylated and subsequently oxidized to the 11-ketones. The product obtained from the more polar hydroxyester (XXIXB) was found to be identical to the acetoxyester (XXXIB) of known configuration. The remaining less polar hydroxyester (XXIXA) must therefore have the α-configuration at C-20. Upon comparing the n.m.r.-spectrum of the esters (XXIXA) and (XXIXB) it was noticed that the position of the signal of the C-20 proton and the magnitude of its coupling constant with the adjacent proton at C-17 could provide a general method for characterizing these epimers.

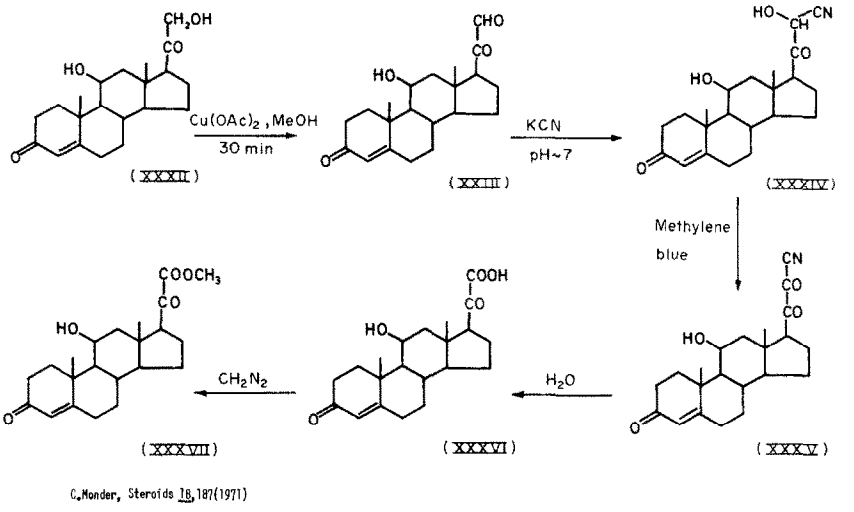
The C-20 proton of the 20α-alcohol (XXIXA) appears as a doublet at $\delta = 4{\cdot}15$ ppm with a coupling constant of 3·5 Hz, whereas for the 20β-alcohol



H-18 an H-20 NMR data of 20-hydroxy-pregnan-21-oic acid esters*

	X	Y	Z	R	δ H-18 (A)	δ H-18 (B)	δ H-20 (A)	δ H-20 (B)
	H	H	H	CH_3	1.06	1.09	4.15 (J = 3.5 Hz)	4.07 (J = 9.5 Hz)
	CH_3	H	H	C_6H_9	1.08	1.11	4.20 (J = 3.5 Hz)	4.12 (J = 9 Hz)
	CH_3	F	H	C_6H_9	1.06	1.10	4.17 (J = 3.5 Hz)	4.09 (J = 9 Hz)
Δ^1	CH_3	H	H	C_6H_9	1.10	1.12	4.16 (J = 3.5 Hz)	4.10 (J = 9 Hz)
Δ^1	CH_3	F	H	CH_3	1.10	1.12	4.19 (J = 3.5 Hz)	4.13 (J = 9 Hz)
Δ^1	CH_3	F	F	CH_3	1.10	1.12	4.19 (J = 3.5 Hz)	4.13 (J = 9 Hz)

* Chemical shift δ in ppm with tetramethylsilane as internal standard in $CDCl_3$



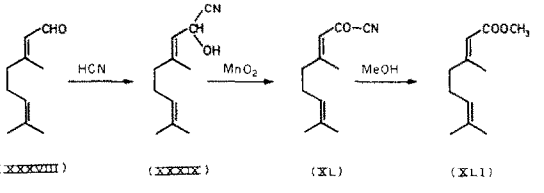
C. Monder, Steroids 18, 187(1971)

(XXIXB) the corresponding signal appears at $\delta =$ 4·07 ppm with a coupling constant of 9·5 Hz.

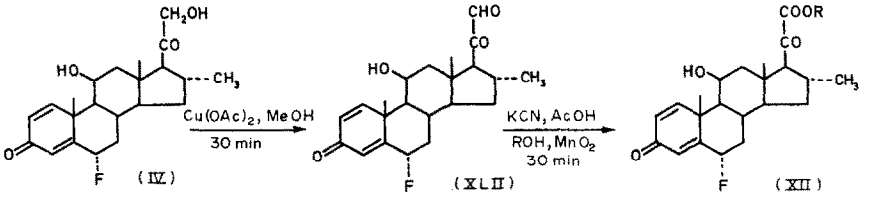
Investigation of all 20-hydroxyesters prepared by us has shown this phenomenon to be of general significance. For every pair of alcohols it is found that the less polar isomer (series A) in all instances exhibits a coupling constant of 3·5 Hz whereas for the more polar isomer (series B) a larger coupling constant of 9 Hz is found. On this basis, the A- and B-series are assigned the 20α- and 20β-configuration, respectively. The n.m.r. signals of the 18-methyl groups are, however, unsuitable for assignment of configuration as in both series there are observed very slight differences in the chemical shifts of these protons.

In 1971 Monder[10] described the synthesis of corticosterone-21-acid methyl ester (XXXVII) by an alternative oxidation process. The aldehyde (XXXIII), prepared in the usual manner, was converted to the cyanohydrin (XXXIV) which was further oxidized with methylene blue to the very reactive cyanoketone (XXXV). Solvolysis of this intermediate to the acid (XXXVI) proceeds very rapidly with water and subsequent esterification with diazomethane yields the ester (XXXVII). The disadvantage of this method lies in the fact that the rather sensitive ketoacid has to be isolated before being converted to the ester.

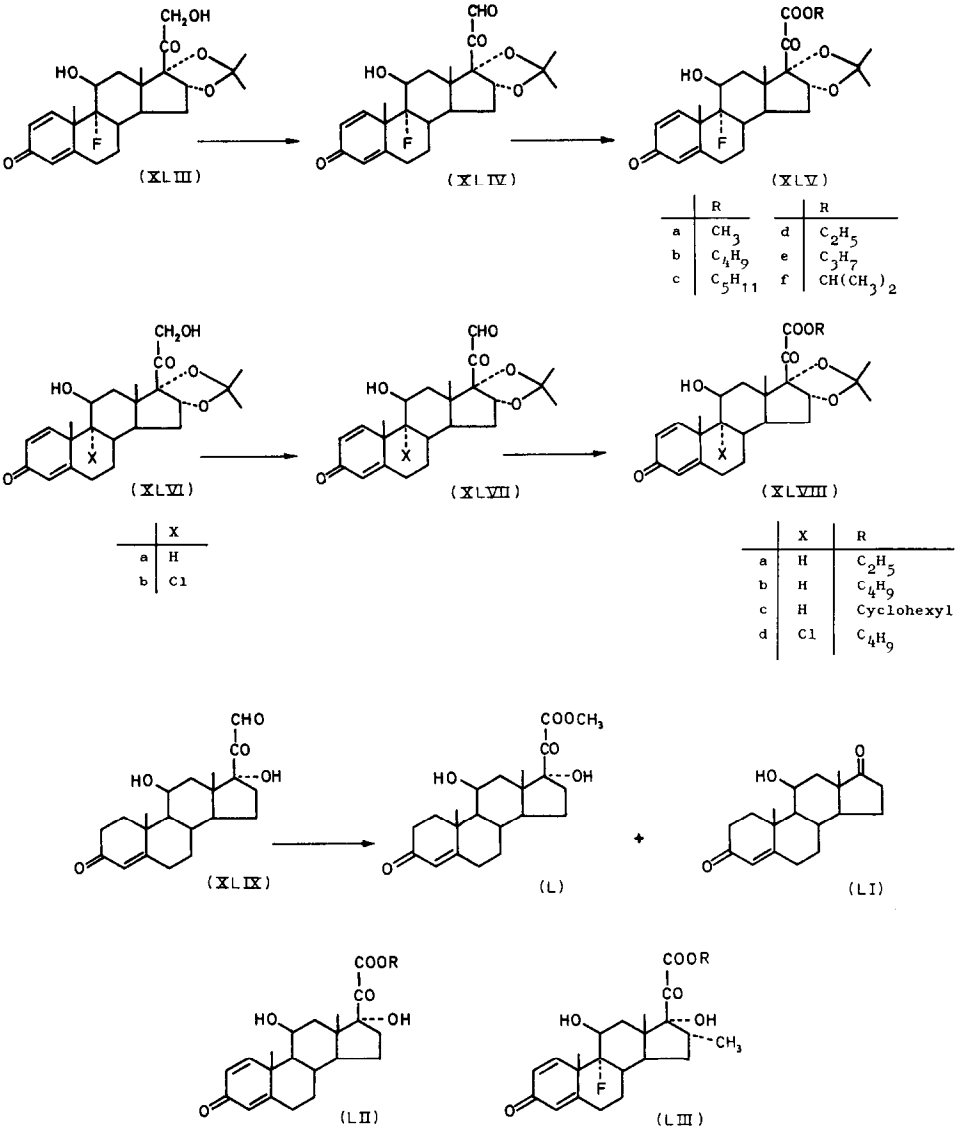
In this connection, the oxidative esterification of α,β-unsaturated aldehydes reported by Corey and coworkers [11] was also of interest. By this method it was possible to convert geranial (XXXVIII) directly to methyl geraniate (XLI) with manganese dioxide in the presence of sodium cyanide, methanol and acetic acid. The reaction proceeds through the intermediate cyanohydrin (XXXIX) which is oxidized to the cyanoketone (XL) with active manganese dioxide. The cyanide ion is displaced by methanol with subsequent formation of the ester (XLI). The time of reaction in this specific instance is reported to be 12 h and the ester is obtained in 90% yield.



We found that this method was also suitable for the synthesis of α-ketoesters from the corresponding ketoaldehydes. The aldehyde (XLII), prepared in the usual fashion by cupric acetate oxidation of fluocortolone (IV), was smoothly converted to the fluocortolone acid methyl ester (XIIa) utilizing the Corey method. Substitution of butanol or isopropanol for methanol made it possible to prepare directly the corresponding butyl and isopropyl esters. The reaction was complete after 30–90 min, that is, approximately 10–20 times as fast as the conversion of the



	R
a	CH_3
b	C_4H_9
c	$CH(CH_3)_2$



α,β-unsaturated aldehyde to the corresponding ester. The yield of crystallized product amounts to 75% based on fluocortolone as starting material.

With this method we were then able to prepare the ester derivatives of α-ketoacids in the 16α,17-isopropylidenedioxy series. Triamcinolone acetonide (XLIII) could be smoothly converted into its methyl, butyl and pentyl esters (XLVa–c). The ethyl, propyl and isopropyl esters (XLVd–f) were obtained by transesterification of the originally-formed pentyl ester (XLVc). Likewise, the 9-desfluoro- and 9-chloro-derivative of triamcinolone (XLVI) were converted into various 21-acid esters (XLVIII).

In contrast, however, the oxidative esterification of cortisol aldehyde (XLIX) or, in general, derivatives possessing a 17-hydroxy group, proceeded with poor yields under the usual conditions. Instead, side chain degradation predominated with consequent formation of the 17-ketone. After further experimentation the reaction conditions could be refined so that the side chain remained intact during the oxidation. The 21-acid esters of prednisolone and dexamethasone (LII) and (LIII) and of other 17-hydroxycorticoids could then be synthesized by this method.

In conclusion, the syntheses I have presented during this lecture have all been directed to the preparation of a new class of locally active, anti-inflammatory corticoids. We are hopeful that a few of these compounds will be responsible for an advance in the therapy of inflammatory and allergic dermatoses.

Acknowledgement—The authors wish to thank Dr. D. Rosenberg for the measurement and analysis of the n.m.r. data.

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