a methylene (7.92 and 7.70 τ) adjacent to a quarternary carbon. The diol (Ⅳ) was oxidized with chromic acid to give the original dione, curdione, and the ketol (Ⅴ), $C_{15}H_{26}O_2$, m.p. 70~71°, $[\alpha]_D$ −303°, IR (CCl_4): ν_{O-H} 3410, $\nu_{C=O}$ 1688 cm^{-1}, NMR: 1H triplet at 6.78 τ (unresolved, J=11, H-C≤OH). Reduction of curdione with sodium borohydride afforded the other ketol (Ⅵ), $C_{15}H_{26}O_2$, $[\alpha]_D$ +74.5°, IR (liquid): ν_{O-H} 3500, $\nu_{C=O}$ 1696 cm^{-1}, NMR: 1H triplet at 5.88 τ (unresolved, J=4, H-C≤OH) which was regenerated to curdione on chromic acid oxidation. These observations indicate that curdione is a dione.

The presence of the above functions and the molecular formula require curdione to be a monocarbocyclic compound. When the diol (Ⅳ) was dehydrogenated with palladized charcoal guaiazulene (Ⅶ) was formed; a fact which confirmed that curdione has the germacrane skeleton.

Alkali treatment of the dihydro-derivative (Ⅲ) resulted in the aldol condensation to give the cyclopentenone (Ⅷ), $C_{15}H_{24}O$, UV λ_{max}^{EtOH} 243 mμ (ε 12500), IR (liquid): $\nu_{C=O}$ 1694, $\nu_{C=C}$ 1639 cm^{-1}, NMR: no vinyl hydrogen. The enone (Ⅷ) was hydrogenated to afford the cyclopentanone (Ⅸ), IR (liquid): $\nu_{C=O}$ 1733 cm^{-1}, while reduction with lithium aluminum hydride gave a hydrocarbon, NMR: no vinyl hydrogen, which on dehydrogenation with palladium-on-carbon furnished vetivazulene (Ⅹ). This series of reactions established the positions of the carbonyl groups of curdione being situated at C-5 and C-8 in the germacrane skeleton. From the ultraviolet (λ_{max}^{EtOH} 299 mμ (ε 250)) and NMR evidence, the only remaining functional group, the trisubstituted ethylenic linkage, must consequently be located at C-1:C-10 in curdione.

Curdione is thus elucidated to be as shown in formula Ⅰ.

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Direct Analysis of Corticosteroids by Gas Chromatography as Trimethylsilyl Ethers of Methyloximes

In the gas chromatographic analysis of corticosteroids, it has been recognized that all of the known methods for preparing derivatives with favorable volatility, such as trimethylsilylation of a hydroxyl group[1] and a very recently reported methoxyimination of a carbonyl function,[2] do not prevent the 17β-side chains from undergoing pyrolytic cleavage. Therefore, the analysis of corticosteroids by gas chromatography has been carried out after their quantitative conversion to the corresponding 17-ketosteroids with some appropriate oxidant.[3] In this case, however, the presence of 17-ketosteroids in the original mixture interferes with the analysis of the corticosteroids.

1) T. Luukkainen, W. J. A. VandenHeuvel, E. D. A. Haahti, E. C. Horning: Biochim. Biophys. Acta, **52**, 599 (1961).
2) H. M. Fales, T. Luukkainen: Anal. Chem., **37**, 955 (1965).
3) I. Merits: J. Lipid Research, **3**, 126 (1962).

In the present paper it will be shown that nine corticosteroids can be directly analyzed by gas chromatography without any pyrolysis after their conversion to the corresponding methyloximes followed by trimethylsilylation.*1

Quantitative formation of chemically stable methyloxime derivatives of ketosteroids was carefully confirmed by silicagel thin-layer chromatography (Wakogel B-O, benzene/acetone 8:1), and usually required no longer than three hours at room temperature, except in the case of 11-ketosteroids, which have been previously reported to resist the formation of the methyloximes even under highly drastic conditions.[2] The delay in methyloxime formation of the dihydroxyacetone moiety is probably due to steric hindrance to the 20-oxo group by two adjacent hydroxyl groups.

On the thin-layer chromatogram the resultant oxime of each corticosteroid gave a clearly distinguishable pair of spots corresponding to its *syn* and *anti* isomers. In this connection, a previous report from this laboratory has shown that an α,β-unsaturated aldoxime of a steroid gives two geometrical isomers which can be separated on a thin-layer chromatogram.[4] When a methyloxime of testosterone containing an α,β-unsaturated ketone moiety was used as a model compound, it also afforded two spots having much higher Rf values than the original steroid, whereas the reaction mixture showed only a single peak on the gas chromatogram.*2 In addition, extraction of each geometrical isomer of the testosterone methyloximes from the silicagel plate followed by application of gas chromatography*2 showed that they have the same retention time.

After the complete formation of the corticosteroid methyloxime the solvent of the reaction mixture was evaporated with a nitrogen gas stream at room temperature, and its trimethylsilyl derivative was prepared in the usual way[1] whereby the reaction was carried out by allowing to stand overnight. In this case the progress of the reaction was also checked by thin-layer chromatography, and it was found that complete formation of the trimethylsilyl ether of a steroid with a dihydroxyacetone moiety required a somewhat longer reaction time than the ordinary steroidal alcohols; for instance, the methyloxime of cortisone was not quantitatively converted to the corresponding trimethylsilyl ether even after three hours. On the chromatogram the trimethylsilyl ether of each corticosteroid methyloxime also gave a pair of spots due to the original isomeric oximes. The solvent of the reaction mixture was evaporated with a dry nitrogen gas stream at room temperature and the residue was dissolved in anhydrous hexane to make a 1% solution of the trimethylsilyl ether of the methyloxime, which was analyzed on the Shimadzu Model GC-1C Gas Chromatograph using a hydrogen flame ionization detector. The pyrex glass column, which was packed with 1.5% SE-30 on 60~80 mesh Chromosorb W, was 180 cm. long with a 4 mm. internal diameter. The temperature of the column during analysis was 235° and the detector was at 250°. The carrier gas, nitrogen, was adjusted to a flow of 35 ml./min.

*1 After preparation of this manuscript, we knew that a preliminary note concerning the gas chromatographic analysis of C_{19} and C_{21} human urinary steroids had been published (W. L. Gardiner and E. C. Horning: Biochim. Biophys. Acta, **155**, 524 (1966)), in which their method used for the preparation of the derivatives of the steroids is based on the same idea as described in our paper. Although they concluded that the hydroxyl group at both 11β and 17α positions did not be trimethylsilylated, from results of gas chromatograph-mass spectrometric analysis of the trimethylsilyl ethers of tetrahydrocorticosteroid methyloximes, our unpublished data indicated that if the 17α-hydroxyl group of the corticosteroid methyloximes is not trimethylsilylated, their 17β-side chains suffer a pyrolytic cleavage to the corresponding 17-ketosteroids, and also that the steroids with a 11β-hydroxyl group are easily converted to the corresponding trimethylsilyl derivatives, which were confirmed by the thin-layer and the gas chromatographies.

*2 Conditions used are same as the analysis of the trimethylsilyl ethers of the corticosteroid methyloximes.

4) S. Hara, K. Oka: Tetrahedron Letters, **1966**, 1057.

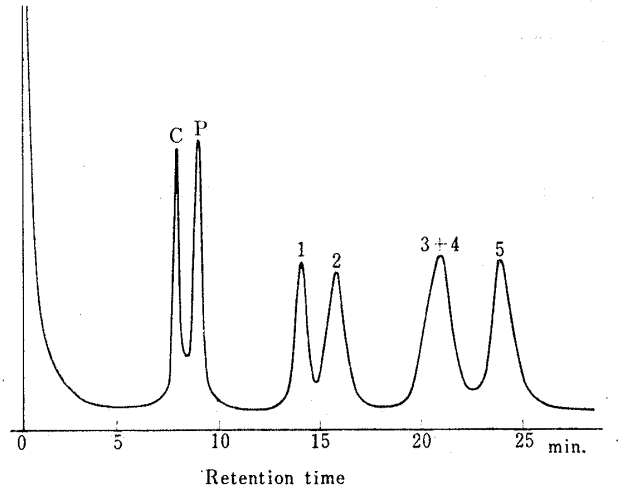

Fig. 1. Gas Chromatogram of Trimethylsilyl Derivatives of Corticosteroid Methyloximes

C : cholestane P : progesterone
1 : desoxycorticosterone
2 : 17α-hydroxydesoxycorticosterone
3 : cortisone 4 : corticosterone
5 : cortisol

Table I shows the relative retention time (RRT) values of the trimethylsilyl ethers of the methyloximes of nine corticosteroids and a methyloxime of progesterone to that of cholestane as an internal reference; five are naturally occurring steroids and the other four are their simple derivatives used widely in clinical fields. From the data, it is expected that a mixture of these corticosteroids should be successfully separated if two of the three steroids, cortisol, prednisone and prednisolone as well as either cortisone or corticosterone are excluded from the mixture. Actually, a simultaneous analysis of a mixture of five naturally occurring corticosteroids gave four well separated peaks on the gas chromatogram plus two peaks corresponding to cholestane and the methyloxime of progesterone, injected as internal references (Fig. 1.).

For the purpose of examining the additive effect of each functional group on the RRT, its logarithmic value (log RRT, Table I) was taken and the effect of a functional group was represented as a difference (Δ log RRT) between log RRT values of compounds with and without the given functional group, as reported in the preceding paper.[5] The date summarized in Table II show that replacement of a hydrogen atom attached to the 11β-, 17α- or 21-position of the progesterone skeleton with a trimethylsilyloxy group gives rise in each case to a different and somewhat longer retention time (positive Δ log RRT). However, because the replacement at the same position gave very similar Δ log RRT values, the establishment of the additivity rule was confirmed.

Table I. Relative Retention Time of Trimethylsilyl Ethers of Corticosteroid Methyloximes

No.	Compound	1.5% SE-30	
		RRT[a]	log RRT
1	21-hydroxypregn-4-ene-3,20-dione (desoxycorticosterone)	1.80	0.255
2	17α,21-dihydroxypregn-4-ene-3,20-dione (17α-hydroxydesoxycorticosterone)	2.01	0.303
3	17α,21-dihydroxypregn-4-ene-3,11,20-trione (cortisone)	2.59	0.413
4	11β,21-dihydroxypregn-4-ene-3,20-dione (corticosterone)	2.69	0.430
5	11β,17α,21-trihydroxypregn-4-ene-3,20-dione (cortisol)	3.03	0.481
6	17α,21-dihydroxypregna-1,4-diene-3,11,20-trione (prednisone)	3.06	0.486
7	11β,17α,21-trihydroxypregna-1,4-diene-3,20-dione (prednisolone)	3.04	0.483
8	21-acetoxy-17α-hydroxypregn-4-ene-3,11,20-trione (cortisone acetate)	3.26	0.513
9	21-acetoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione (cortisol acetate)	3.56	0.551
	pregn-4-ene-3,20-dione (progesterone)[b]	1.12	0.049

a) Relative retention time to that of cholestane (7.86 min.) as an internal reference. These are mean values obtained by at least five times analyses a sample. The amount of each sample is 5 to 10 μg.
b) A mother compound for calculation of substituent effect.

5) S. Hara, T. Watabe, Y. Ike, N. Ikekawa : to be published.

Table II. Δ Log RRT of Converted Functional Group of Corticosteroids

Converted functional group	Compound No.	Δ Log RRT
21-OTMSi	1～P[a)]	0.206
17α-OTMSi	2～1	0.048
	5～4	0.051
11=O	3～2	0.110
21-OTMSi ⟶ 21-OAc	9～5	0.070
	8～3	0.100

a) progesterone

Utilization of the Δ log RRT value would have the following advantages : a) prediction of the RRT values of given corticosteroids, and b) identification of corticoids from their RRT values.

Making use of this general procedure for protecting the side chains of corticosteroids, further investigations concerning the analysis of other synthetic steroids and corticosteroids contained in biological materials are in progress.

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Oxypyrrolnitrin : A Metabolite of *Pseudomonas*

In studies on the metabolites of *Pseudomonas*, 3-chloro-4-(2-nitro-3-chlorophenyl)-pyrrole[1] and 2,3-dichloro-4-(2-nitrophenyl)pyrrole[2] were found to exist. Further investigations showed a coexistence of oxypyrrolnitrin in the same cultural broth. The existence of these metabolites are easily recognized by the characteristic color reaction using Ehrlich reagent.

The acetone extract of this bacterial cell was purified by column chromatography using silica gel and benzene-chloroform, and pale yellow rhombic crystals were obtained by recrystallization from ether-hexane solution; (I), m.p. 215～216°(dec.), mol. wt. 273 by mass spectrometry, $C_{10}H_6O_3N_2Cl_2$ (*Anal.* Calcd. : C, 43.99; H, 2.22; N, 10.26. Found : C, 43.93; H, 2.18; N, 10.26), UV λ_{max}^{EtOH} mμ (log ε) : 290 (3.54). The infrared spectrum of this substance showed the presence of the following functional groups; -NH (3300 cm^{-1}), -OH (3475, 1193 cm^{-1}) and $-NO_2$ (1530, 1365 cm^{-1}).

1) Pyrrolnitrin : K. Arima, H. Imanaka, M. Kousaka, A. Fukuta, G. Tamura : Agr. Biol. Chem., **28**, 575 (1964).
2) Isopyrrolnitrin : M. Hashimoto, K. Hattori : Bull. Chem. Soc. Jap., **39**, 410 (1966).