Extraction of $C_{21}O_5$ and $C_{21}O_6$ Steroids from Aqueous Media

Although a great variety of solvents have been used for the extraction of corticosteroids, it seems, as has been pointed out by Mason (*1*), that "solvents have been chosen as a matter of personal preference rather than on the basis of any study of their merits and shortcomings." By and large, the chlorinated hydrocarbons have become the favorite solvents after the introduction of ethylene chloride as a selective solvent for cortical hormones by Cartland and Kuizenga (*2*). Although Reichstein (*3*) indicated the excellent extraction properties of ethyl acetate for corticosteroids, relatively little use has been made of this solvent until quite recently, when Meyer (*4*), after testing a variety of solvents for extraction of blood, found that ethyl acetate was particularly favorable, since it extracted smaller amounts of extraneous material and did not form emulsions.

Owing to its relative nonemulsifying properties, as discovered by Meyer, ethyl acetate was subsequently introduced for extraction of corticosteroids from guinea pig and human urine (*5–7*). In these studies, in which the more polar solvent ethyl acetate was used, the two $C_{21}O_6$ steroids 6β-hydroxycortisol and 2α-hydroxycortisol have been isolated. In this connection, the finding of corticosteroids with mobilities on paper of $C_{21}O_6$ steroids in adrenal extracts obtained by the use of ethyl acetate as the extracting solvent is noteworthy (*8*). The fact that in previous studies, in which methylene chloride was used, these $C_{21}O_6$ steroids were not observed except in trace amounts (*9*) has made it pertinent to study the extractability of $C_{21}O_6$ steroids from aqueous media.

In this report (*10*) partition coefficients of some $C_{21}O_5$ and $C_{21}O_6$ steroids for the two systems chloroform/water and ethyl acetate/water are presented (Table 1). The partition coefficients (expressed as $K = C_{org.}/C_{aq.}$) were determined by shaking the solvents containing the steroids (100 to 600γ) with an equal volume of water (5 ml). Solvents that were not previously saturated with respect to each other were used, since the partition coefficients were required for calculations on batch extraction with new solvent. The partitions were done at room temperature. Determinations were done on aliquots from each phase.

It appears from Table 1 that ethyl acetate is a better extracting solvent than chloroform for both the $C_{21}O_5$ and $C_{21}O_6$ steroids studied. From the partition coefficients of the $C_{21}O_6$ steroids given, it becomes clear that chloroform (similar data were obtained with methylene chloride) is not a suitable extraction solvent for these steroids. Since in the usual extraction procedure the original extract is further purified by washing with alkali and water to remove acidic interfering material, only small yields of $C_{21}O_6$ steroids can be obtained owing to the appreciable extraction by the water phase.

In view of the relatively low partition coefficients of some $C_{21}O_5$ steroids for the $CHCl_3/H_2O$ system, a reevaluation of the significance of earlier quantitative studies in which chlorinated hydrocarbons have been used will be necessary. Thus, the relatively small yields of 3α, 11β, 17α, 21-tetrahydroxypregnan-20-one isolated from human urine following cortisol feeding undoubtedly have been the result of the aqueous washes of the methylene chloride extracts during the extraction and fractionation procedure (*9*). The present data indicate the necessity for further work on extraction and separation of highly hydrophilic steroids, since increasing the polarity of the solvents decreases the extraction selectivity and presents greater difficulties in isolation.

Table 1. Partition coefficients of some $C_{21}O_5$ and $C_{21}O_6$ steroids.* The determinations were done by ultraviolet spectrophotometry in methanol with the Beckman DU (UV), by the phosphomolybdic acid (P.M.), or the Porter-Silber (P.S.) reactions as indicated.

No.	Steroid	K $CHCl_3/H_2O$	K EtOAc/H_2O	Determination method
1.	11β, 17α, 21-Trihydroxy-4-pregnene-3,20-dione	6.4	12.2	UV at 240 mμ; P.M. and P.S.
2.	11β, 17α, 21-Trihydroxy-4,6-pregnadiene-3, 20-dione	4.8	12.6	UV at 280 mμ
3.	11β, 17α, 21-Trihydroxy-1,4-pregnadiene-3, 20-dione	3.6	11.2	UV at 242 mμ
4.	3α, 11β, 17α, 21-Tetrahydroxy-pregnan-20-one†	2.4	11.0	P.M.
5.	11β, 17α, 20β, 21-Tetrahydroxy-4-pregnen-3-one	1.1	1.6	UV at 240 mμ
6.	11β, 17α, 20α, 21-Tetrahydroxy-4-pregnen-3-one	0.9	1.7	UV at 242 mμ
7.	6β, 17α, 21-Trihydroxy-4-pregnene-3,11,20-trione	0.3	2.2	UV at 233 mμ
8.	9α, 11β, 17α, 21-Tetrahydroxy-4-pregnene-3,20-dione	0.1	2.0	UV at 242 mμ
9.	2α, 11β, 17α, 21-Tetrahydroxy-4-pregnene-3,20-dione	0.3	1.9	P.S.
10.	6β, 11β, 17α, 21-Tetrahydroxy-4-pregnene-3,20-dione	0.05	0.9	P.S.

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† For unexplained reasons, variable results were obtained with steroid 4 during the chloroform-water partitions with low recoveries from the aqueous phase. The value given in the table represents the average of three determinations in which the total recoveries were above 90 percent.

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References and Notes

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