5. Kuhn, R. and Brossmer, R. *Chem. Ber.* **87** (1954) 123.
6. Zilliken, F., Braun, G. A. and György, P. *Arch. Biochem. and Biophys.* **54** (1955) 564.
7. Odin, L. *Acta Chem. Scand.* **9** (1955) 714.
8. Odin, L. *Acta Chem. Scand.* **9** (1955) 862.
9. Sørensen, M. *Biochem. Z.* **269** (1934) 271.
10. Odin, L. *Acta Chem. Scand.* **5** (1951) 1420.
11. Werner, I. and Odin, L. *Acta Soc. Med. Upsaliensis* **57** (1952) 230.
12. Freudenberg, K., Molter, H. and Walch, H. *Sitzber. heidelberg. Akad. Wiss., Math.-naturw. Kl. 9. Abh.* (1940).
13. Ross, V., Moore, D. H. and Miller, E. G. *J. Biol. Chem.* **144** (1942) 667.
14. Gardell, S. *Acta Chem. Scand.* **7** (1953) 207.
15. Gottschalk, A. and Lind, P. E. *Brit. J. Exptl. Pathol.* **30** (1949) 85.

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Preparation of 2,5-Dimethyl-1,4-Dioxan

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In a recent paper it has been shown that Levene and Walti's method for the preparation of 2,6-dimethyl-1,4-dioxan yielded the cyclic acetal 2-ethyl-4-methyl-1,3-dioxolan [1]. 2,6-Dimethyl-1,4-dioxan, however, has been prepared by Nesmeyanov and Lutsenko [2]. In the course of studies related to addition compounds of ethers, which are being carried out in this laboratory it was found desirable to prepare 2,5-dimethyl-1,4-dioxan as well. Only one previous work is reported on the preparation of this compound. I. G. Farbenindustrie in a patent states that the compound is formed when propylene oxide vapours are passed over sodium bisulphate or other acidic substances at elevated temperatures [3]. The sodium bisulphate reaction, however, when repeated here was found to give a mixture of compounds.

Looking for another starting material for the preparation of pure 2,5-dimethyl-1,4-dioxan we discovered that *trans*-2,5-bis-(iodomethyl)-1,4-dioxan has been prepared through a few intermediates from allyl alcohol and mercuric nitrate [4]. This compound should be expected to give 2,5-dimethyl-1,4-dioxan on reduction.

However, experiments showed that the dioxan ring may be subject to easy cleavage under reducing conditions. By the use of zinc and ethanol or zinc and acetic acid as reducing agents allyl alcohol or allyl acetate were formed, respectively. This difficulty was overcome by using lithium aluminium hydride, which yielded the desired product. Physical constants of the purified reaction product: B.p. (750 mm) 121.5°; m. p. −4.5°; mol.wt. 113.9 (calc. for dimethyldioxan 116.2); d_4^{22} 0.932; n_D^{22} 1.4147; mol. refraction 30.61 (calc. 30.99).

1,4-Dioxan is known to form addition compounds with numerous inorganic salts. An X-ray structure investigation of the mercuric chloride addition compound has been carried out by Hassel and Hvoslef [5].

The 2,5-dimethyldioxan prepared in the present work forms a similar addition compound with mercuric chloride. An X-ray structure investigation of this addition compound has been started in order to confirm that the methyl groups are mutually "*trans*" situated.

Experimental. To 63 g of *trans*-2,5-bis-(iodomethyl)-1,4-dioxan prepared according to Summberbell and Stephens [4] were added 1 l of ether and 20.5 g of lithium aluminium hydride. The round bottom flask was equipped with a sealed stirrer and reflux condenser with a drying tube on top. The stirring was continued with gentle boiling for 8 days. The actual reaction time is probably shorter, but in a preliminary test experiment unreacted starting material was recovered after several days. Finally water was added drop by drop until the evolution of hydrogen had ceased. The etheral layer was separated from the precipitate, the latter washed several times with ether and the ether solutions combined. The main part of the ether was removed by distillation, rest *ca.* 200 ml. To remove iodine the solution was shaken until it became colourless with a few ml of a saturated aqueous sodium thiosulphate solution. After drying over anhydrous sodium sulphate the ether was distilled off and the remaining liquid distilled in a 24 inches Podbielniak column with a reflux ratio of 50 : 1. The liquid distilled at constant temperature .Yield *ca.* 15 ml. (Found: C 61.89; H 10.34. Calc. for $C_6H_{12}O_2$: C 62.01; H 10.41).

1. Augdahl E. and Hassel O. *Acta Chem. Scand.* **9** (1955) 172.
2. Nesmeyanov A. and Lutsenko I. *Bull. acad. sci. U.S.S.R. Classe sci. chim.* **1943** 296—304; *Chem. Abstracts* **38** (1944) 5499 $_1$.
3. I. G. Farbenindustrie D.R.P. 613.261 *Chem. Abstracts* **29** (1935) 5464 $_2$.
4. Summberbell, R. and Stephens, J. *J. Am. Chem. Soc.* **76** (1954) 6404.
5. Hassel, O. and Hvoslef, J. *Acta Chem. Scand.* **8** (1954) 1953.

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Analysis of Steroids in Dog Adrenal Vein Blood by Countercurrent Distribution

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Besides Δ^4-pregnene-11β, 17a, 21-triol-3,20-dione (cortisol) and Δ^4-pregnene-11β, 21-diol-3,20-dione (corticosterone) three new, very nonpolar ketonic steroids have been demonstrated in appreciable amounts in adrenal vein blood of dogs, two of them being Δ^4-3-keto-steroids and one of these having at least one hydroxyl group.

The separation of pure corticosteroids by countercurrent distribution has been reported elsewhere [1]. Adrenal vein blood was collected from anaesthetized dogs held at constant blood pressure. The blood was cooled during the collection and thereafter frozen to −20° C. After rapid thawing it was diluted with distilled water (1:1) and extracted with ethyl acetate by carrying out a fine droplet distribution of the blood into the extracting medium. The dried extract was distributed twice between *n*-hexane and 50 % ethanol in water. The combined lower phase is expected to contain all conventional corticosteroids possibly present, since their partition coefficients are less than 0.09 in this system. Different components with specific extinction at 240 mμ were separated by countercurrent distribution using 24 transfers and a sequence of different solvent systems (Table 1). The upper phase of the original distribution was subjected to a micro-Girard separation [2]. Countercurrent distribution of the ketonic fraction (Table 2) revealed the presence of two compounds with extinction maximum at 240 mμ (designed x and z). The x-component was acylable as the partition coefficient shifted from 0.67 to 6.7 in the same system after treatment with acetyl chloride (Table 2). The blue tetrazolium [3], Porter-Silber [4] and Zimmermann [5] reactions were negative. The last reaction was negative after oxidation with sodium bismuthate [6] as well. A third component (designed y) appeared having an extinction maximum between 276 and 280 mμ. The Kober [7], blue tetrazolium and Porter-Silber reactions were negative, but a sharp extinction peak appeared at 260—265 mμ with the phenylhydrazine-sulfuric acid reagent. This was

Table 1.

No.	Countercurrent distribution (CC) Solvent system	Followed by CC No.	Partition coefficients (K) of polar components in adrenal vein blood: K	*Steroid identified*
1:	Benzene/H_2O	2	0.35	
		3	11.5	
2:	20 % Ethanol + 80 % H_2O / 50 % $CHCl_3$ + 50 % *n*-hexane		2.57	Cortisol
3:	60 % *n*-Hexane + 40 % benzene	4	0.70	
	5 % Ethanol + 95 % H_2O	5	11.5	
4:	20 % Ethanol + 80 % H_2O / CCl_4		1.6	Corticosterone
5:	*n*-Hexane / H_2O		1.8	Not identified Only a small amount