PAPER CHROMATOGRAPHIC CHARACTERIZATION OF SOME PREGNENE ALCOHOLS, THIOLS, AMINES, AND DERIVATIVES

LELAND L. SMITH*

Chemical Process Improvement Department,
Lederle Laboratories Division, American Cyanamid Company,
Pearl River, N.Y. (U.S.A.)

(Received August 21st, 1961)

INTRODUCTION

With the recognition that 17α,21-dihydroxy-20-ketosteroids are converted microbiologically to 21-acetylamino-17α-hydroxy-20-ketones[1] an interest developed in the possibilities of detecting other such reactions in biological systems. Conversion of the 21-hydroxyl group to other 21-amino derivatives, 21-thiol derivatives, etc. was conceived, and suitable means of recognition of these types by paper chromatography were sought. The present paper deals with the chromatographic properties of several 21-acetylamino-, 21-amino-, 21-thio-, 21-acetylthio-, 21-methylthio-pregnenes in comparison with related oxygen analogs.

EXPERIMENTAL

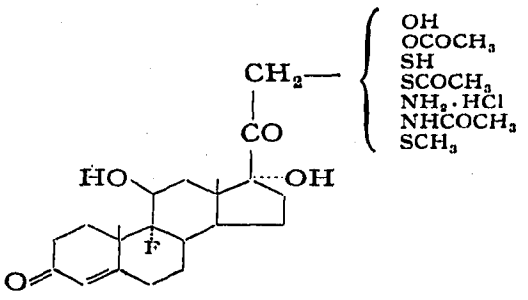
The paper chromatographic examinations were conducted using Bush-type solvent systems previously described[2]. Two previously undescribed solvent systems, system VII, petroleum ether (b.p. 90–100°)–methanol–water (10:7:3) and system VIII, benzene–petroleum ether (b.p. 90–100°)–methanol–water (5:15:14:6), were used for the deoxycorticosterone derivatives. Steroids were detected via ultraviolet light absorption on paper, alkaline tetrazolium blue, and isonicotinic acid hydrazide[3].

Micro-acetylations on Whatman No. 1 filter paper were conducted using 20 μg of steroid applied in 10 μl of methanol, air-dried, spotted over twice with 10 μl of an acetic anhydride–pyridine (1:3) solution[4]. After 10 min of air drying the paper was equilibrated and irrigated in the usual manner using the selected solvent system. Known acetylated steroids were run (untreated) on the same sheet of paper as controls for identification.

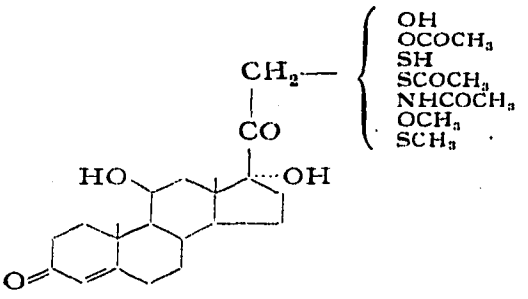
RESULTS

The paper chromatographic mobilities of several hydrocortisone, 9α-fluorohydrocortisone, and deoxycorticosterone derivatives are presented in Table I. The analogous derivatives all contain as a common structural feature the pregnene nucleus and associated functional groups, the only structural difference being that at the C-21 position.

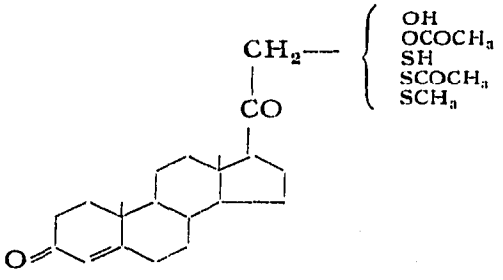
* Present address: Wyeth Laboratories, Inc., Philadelphia, Pa., U.S.A.



9α-Fluorohydrocortisone derivatives



Hydrocortisone derivatives



Deoxycorticosterone derivatives

TABLE I

COMPARISON OF PAPER CHROMATOGRAPHIC MOBILITIES OF STEROIDAL OXYGEN, SULFUR, AND NITROGEN DERIVATIVES

Steroid nucleus	*System*	*R_F of parent type*			*R_F of acetyl derivatives*			*R_F of methyl ethers*	
		21-OH	*21-SH*	*21-NH_2*	*21-OAc*	*21-SAc*	*21-NHAc*	*21-OMe*	*21-SMe*
9α-Fluorohydrocortisone	I	0.59	0.90	0.04	1.00	1.00	0.66		0.94
	II	0.80	0.95	0.05	1.00	1.00	0.67		0.95
	III	0.63	0.91	0.03	0.90	0.94	0.62		0.92
	IV	0.41	0.69	0.00	0.95	0.98	0.42		0.94
	V	0.03	0.06	0.00	0.29	0.37	0.05		0.41
	VI	0.03	0.10	0.00	0.19	0.28	0.02		0.26
	VII	0.00	0.00	0.00	0.00	0.00	0.00		0.00
	VIII	0.00	0.00	0.00	0.23	0.31	0.00		0.06
Hydrocortisone	I	0.64	0.90		1.00	1.00	0.66	0.86	0.96
	II	0.84	0.95		1.00	1.00	0.67	0.92	0.98
	III	0.68	0.91		0.92	0.95	0.62	0.80	0.94
	IV	0.51	0.92		0.96	0.98	0.49	0.82	0.98
	V	0.06	0.40		0.36	0.52	0.07	0.15	0.57
	VI	0.02	0.29		0.18	0.35	0.04	0.10	0.40
	VII	0.00	0.00		0.00	0.00	0.00	0.00	0.02
	VIII	0.00	0.00		0.27	0.38	0.00	0.02	0.11
Deoxycorticosterone	VII	0.31	0.40		0.52	0.56			0.76
	VIII	0.51	0.83		0.85	0.91			0.91

Complete characterization of each steroid available in all systems was made; however, R_F data for the very mobile deoxycorticosterone derivatives in systems I–VI are not included in Table I as these steroids were uniformly in the solvent front.

All steroids studied gave characteristic yellow fluorescences with isonicotinic acid hydrazide. Of the 21-oxygenated steroids only the 21-O-methyl ether of hydrocortisone failed to reduce alkaline tetrazolium blue on paper. None of the 21-thiosteroids reduced tetrazolium blue with a sensitivity comparable with that obtained with the oxygen α-ketol analogs, and the reliability of the response was not satisfactory. The 21-acetylamino derivatives do not reduce tetrazolium blue on papergrams, although satisfactory response obtains in colorimetric analyses in solution[1]. The 21-amine, 21-amino-9α-fluoro-11β,17α-dihydroxy-4-pregnene-3,20-dione(hydrochloride) reduces alkaline tetrazolium blue on paper, and a strong yellow color is obtained with 0.2 % ninhydrin in acetone.

DISCUSSION

The thiosteroid analogs, whether free thiol, thiol acetate, or thiomethyl ether, are more mobile than the corresponding oxygen derivatives in the several solvent systems studied. The increased mobility is about the same for hydrocortisone and 9α-fluorohydrocortisone derivatives, but the deoxycorticosterone oxygen and sulfur analogs are more nearly alike in polarity.

The data also suggest that the 21-O-methyl ethers are uniformly less mobile than are the 21-O-acetates, while the 21-S-methyl ethers may be of greater, equal, or less mobility than the analogous 21-S-acetates.

The 21-nitrogen derivatives are substantially more polar than their 21-alcohol/21-thiol and 21-acetate/21-thioacetate analogs. It must be noted that the steroid 21-amine used was a hydrochloride salt; however, other salt forms (hydrobromide, hydroperchlorate, picrate) and neutralized preparations had the same mobility as the hydrochloride.

Little attention has been given to studies of the chromatographic properties of oxygen, sulfur, and nitrogen analogs in general. A recent comparison of papergram mobilities of furan carboxylic acids, thiophene carboxylic acids, and pyrrole carboxylic acids establishes that the thiophene acids were more mobile than the oxygen analogs, and this behavior was interpreted in terms of differing "aromatic character"[5]. It is to be noted that the nitrogen analogs were of intermediate mobility, between the thio- and oxy-analogs.

In other comparisons of chromatographic behavior, cysteine is more mobile than serine in one neutral system (95 % ethanol), less mobile in another (95 % methanol)[6]. Cysteine is less mobile than serine in systems incorporating amines[7]. N-Guanidylcysteine is less mobile than N-guanidylserine in several systems[8].

As a further means of recognition of 21-amines and 21-thiols potentially present in biological systems, the conversion of amine and thiol to their respective acetyl derivatives on filter paper prior to chromatography was investigated. Acetylation of pregnen-21-ols under these conditions, using acetic anhydride and pyridine, affords the 21-O-acetates[4]. Others have reported successful acetylation of primary and secondary amines on filter paper[9].

Acetylation of the 21-amine, 21-amino-9α-fluoro-11β,17α-dihydroxy-4-pregnene-3,20-dione on the start line of a chromatogram led to a mixture of the 21-acetyl-

amino derivative and unaltered amine. Acetylation of the three pregnene-21-thiols also gave a two component pattern on the chromatograms, consisting of unaltered thiol and the 21-thiolacetate. The products of the acetylation were identified on the basis of chromatographic behavior in at least three systems using known acetyl derivatives as reference materials.

This combination of chromatographic mobility data, color test behavior, and micro-acetylation reaction on filter paper followed by chromatography has facilitated our search for alteration products of interest in complex biological systems.

ACKNOWLEDGEMENTS

The author is indebted to Mr. R. E. SCHAUB and Dr. M. J. WEISS for the gift of the several thiosteroids and hydrocortisone 21-O-methyl ether used in this study. The capable assistance of Miss ROBERTA ZIMMERMAN in the preparation of the chromatograms is gratefully acknowledged. Systems VII and VIII herein described were devised by Mr. T. FOELL.

SUMMARY

The paper chromatographic and color test behavior of a series of pregnene 21-alcohols, 21-thiols, and 21-amines and of their methyl ether and acetyl derivatives is recorded.

REFERENCES

1 L. L. SMITH, M. MARX, H. MENDELSOHN, T. FOELL AND J. J. GOODMAN, *J. Am. Chem. Soc.*, in the press.
2 L. L. SMITH, T. FOELL, R. DE MAIO AND M. HALWER, *J. Am. Pharm. Assoc., Sci. Ed.*, 48 (1959) 528.
3 L. L. SMITH AND T. FOELL, *Anal. Chem.*, 31 (1959) 102.
4 T. RAHANDRAHA AND A. R. RATSIMAMANGA, *Bull. soc. chim. biol.*, 38 (1956) 897.
5 J. FRANC, *J. Chromatog.*, 3 (1960) 317.
6 H. K. BERRY, H. E. SUTTON, L. CAIN AND J. S. BERRY, *Univ. Texas Publ.*, No. 5109, May 1 (1951) 22.
7 R. R. REDFIELD, *Biochim. Biophys. Acta*, 10 (1953) 344.
8 M. MOURGUE AND R. BARET, *Bull. soc. chim. France*, (1955) 1224.
9 F. KUFFNER AND T. KIRCHENMAYER, *Monatsh. Chem.*, 92 (1961) 701.