TABLE I—MESTRANOL ASSAY ON ESTROGEN–PROGESTIN TABLETS

Lot	Storage Conditions	Original Granulation U.V. Assay, mcg./Tab.	U.V. Assay After Storage, mcg./Tab.[a]	Colorimetric Assay, mcg./Tab.	GLC Assay[a]
A	6 wk. 100/80	50.7	39.1	50.3	51.4
B	3 mo. 100/80	51.3	42.8	50.1	47.2
C	6 mo. 37°	80.0	71.7	77.8	84.3
D	7.5 mo. R.T.	49.9	43.6	48.8	51.6
E	12 mo. R.T.	56.0	51.2	58.7	55.1
F	13 mo. R.T.	51.5	45.6	50.6	52.2
G	18 mo. R.T.	57.6	48.9	56.5	56.0

[a] Data obtained from *Reference 2*.

did not give so stable a color. However, in all instances the color intensity remained constant for a sufficient length of time (20–30 min.) to allow recording of the spectrophotometric readings.

The relationship between absorbance at 550 mμ and the quantity of mestranol was found to obey Beer's law. The standard deviation for accuracy and precision was found to be ± 1.6% and ± 1.52%, respectively.

The specificity of the method for mestranol in the presence of norethindrone and other tablet excipients is demonstrated by the results shown in Table I. The excipients used in the formulation of the tablets were lactose, cornstarch, magnesium stearate, and polyvinylpyrrolidine. The data in Table I include several samples of formulation stored under various conditions of temperature and humidity. A previous study (2) indicated that mestranol did not undergo any decomposition under these conditions. Hence, the selectivity and sensitivity of this colorimetric method appeared adequate for performing routine studies.

Table I compares the data obtained by the ultraviolet and gas chromatographic methods (2) with the present colorimetric results. It is evident that the color assay compares favorably with the gas chromatographic assay and is superior to the ultraviolet method. The mechanism by which phenol–sulfuric acid reacts with mestranol and other estrogens to give colored products is under investigation.

SUMMARY

A quantitative colorimetric procedure using phenol–sulfuric acid has been developed for the analysis of mestranol in estrogen–progestin tablets. The accuracy and precision of this method are ±1.6% and ±1.52%, respectively. The nonphenolic steroid and tablet excipients do not interfere in the determination.

REFERENCES

(1) Klein, S., James, A., and Tuckerman, M., *J. Am. Pharm. Assoc., Sci. Ed.*, **49**, 314(1960).
(2) Shroff, A. P., and Grodsky, J., *J. Pharm. Sci.*, **56**, 460 (1967).
(3) Slaunwhite, W. R., Engle, L. L., Olmsted, P. C., and Carter, P. J., *J. Biol. Chem.*, **191**, 627(1951).
(4) Boscott, R., *Nature*, **162**, 577(1948).
(5) Huttenrauch, R., and Keiner, I., *Pharmazie*, **20**, 242 (1965).
(6) Sjostrom, E., and Nykanen, L., *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 321(1957).
(7) Kadin, H., Ugolini, M. S., and Roberts, H. R., *J. Pharm. Sci.*, **53**, 1313(1964).
(8) Schulz, E. P., *ibid.*, **54**, 144(1965).
(9) Talmage, J. M., Penner, M. H., and Geller, M., *ibid.*, **54**, 1194(1965).
(10) France, J. T., and Knox, B. S., *J. Gas Chromatog.*, **4**, 183(1966).
(11) Gibbs, H. D., *J. Biol. Chem.*, **72**, 649(1927).
(12) Schmulowitz, J. J., and Wylie, H. B., *J. Lab. Clin. Med.*, **21**, 210(1935).
(13) Talbot, N. B., Wolfe, J. K., MacLachlan, E. A., Karush, F., and Butler, A. M., *J. Biol. Chem.*, **134**, 319(1940).
(14) Bender, A. E., and Wilson, A., *Biochem. J.*, **41**, 423 (1947).
(15) Mitchell, F. L., and Davies, R. E., *ibid.*, **56**, 690 (1954).
(16) Libermann, S., *J. Clin. Invest.*, **31**, 341(1952).
(17) Rehm, C. R., and Smith, J. B., *J. Am. Pharm. Assoc., Sci. Ed.*, **49**, 386(1960).
(18) Urbanyi, T., and Rehm, C. R., *J. Pharm. Sci.*, **55**, 501 (1966).

Communications

Identity of Columbianadin and Zosimin

Sir:

Recently, Nikonov and Baranauskaite (1, 2) reported the isolation of a purportedly new coumarin, zosimin, from the roots of *Zosimia absinthifolia* (Vent) Link. This compound analyzed for the formula $C_{19}H_{20}O_5$ and, on alkaline saponification, yielded *cis*-1,2-dimethylacrylic acid (*i.e.*, tiglic acid) and a hydroxylactone, zosimol (m.p. 156–158°), with the formula $C_{14}H_{14}O_4 \cdot {}^1/_2CH_3OH$. It was recognized that zosimin was isomeric with columbianadin (3, 4), but the apparent divergence in optical activities between the two coumarins and the corresponding hydroxylactones led the authors to the conclusion that they were different and that zosimin was, in fact, a new coumarin. Their decision apparently was confirmed by the fact that zosimin yielded tiglic acid on alkaline saponification. The identity of the hydroxylactone was ascertained by Perel'son *et al.* (5) by both spectral and chemical studies, thus eliminating the several other possible structural candidates [*e.g.*, marmesin (6), lomatin (7), 3′-hydroxy-3′,4′-dihydroxanthyletin (8), *etc.*]. On this basis it was concluded originally that zosimin

TABLE I—ROTATIONS OF COMPOUNDS

Compd.	M. p.	Specific Rotation in $CHCl_3$
Zosimin	119–120° (1, 2)	$[\alpha]_D^{16}$ +272° (c, 1.03)
Columbianetin angelate (*i.e.*, columbianadin)	117–119° (3)	$[\alpha]_D^{16}$ +237.8° (c, 1.0)[a]
	121–122° (3)	$[\alpha]_D^{21}$ +234.5° (c, 1.0)[a]
	118.5–119° (4)	$[\alpha]_D^{26}$ +230.5° (c, 1.0)[a]
		$[\alpha]_D^{26}$ +227° (c, 2.8) (4)
Columbianetin tiglate	107–108° (3)	$[\alpha]_D^{16}$ +250.1° (c, 1.0)[a]
		$[\alpha]_D^{21}$ +246.5° (c, 1.0)[a]
		$[\alpha]_D^{26}$ + 243.9° (c, 1.0)[a]
Zosimol	156–158° (1, 2)[b]	$[\alpha]_D^{16}$ +209.4° (c, 0.74)
Columbianetin	164.5–165° (3)	$[\alpha]_D^{16}$ + 165.1° (c, 1.0)[a]
	162.8–163.3° (4)	$[\alpha]_D^{21}$ +164.5° (c, 1.0)[a]

[a] Measurements carried out on a Perkin-Elmer model 141 polarimeter. [b] The discrepancy between this melting point and that of columbianetin is apparently due to ½ mole of methanol of crystallization in contrast to the anhydrous form of columbianetin.

was the tiglate ester of 2′-*tert*-hydroxyisopropyl-2′,3′-dihydrofuro-4′,5′:8,7-coumarin, (*i.e.*, columbianetin tiglate).[1]

Previous studies (3) had encompassed the synthesis of both the angelate (m.p. 117–119°) and tiglate (m.p. 107–108°) esters of columbianetin, and it was apparent that the tiglate ester was not identical with zosimin on the basis of the melting point discrepancy. Since the reported melting point of zosimin (m.p. 119–120°) was so similar to that of columbianetin angelate, (*i.e.*, columbianadin), it seemed reasonable to suspect that they were one and the same. Furthermore, previous experience in this laboratory with a very similar alkaline saponification of columbianadin (3) indicated that tiglic acid was the product instead of the angelic acid that might have been expected. Such a facile conversion of angelic acid to tiglic acid, however, is not unexpected (10).

The difference in specific rotation reported by us for columbianadin $\{[\alpha]_D^{27}$ + 26.5° (c 1.0, dioxane)$\}$ and that obtained for zosimin $\{[\alpha]_D^{16}$ + 272° (c 1.03, $CHCl_3$)$\}$ appears to be one of the principal reasons for deciding on the nonidentity of the two coumarins. However, it is well known that the specific rotation of a given compound can vary widely when using different solvents. When we took the rotations of columbianadin, columbianetin tiglate, and columbianetin in chloroform in order to obtain a direct comparison with zosimin and zosimol (see Table I), the results showed that the apparent wide discrepancy in specific rotations between columbianadin and zosimin as well as between columbianetin and zosimol was substantially eliminated with identical solvents. Nevertheless, the difference in rotation of columbianetin tiglate and angelate was not great enough to make a decision for the identity of either ester with zosimin. The approximately 34° and 44° higher rotation of zosimin and zosimol over columbianetin angelate and columbianetin, respectively, however, suggested an instrumental difference rather than a real difference since these were the only two structural possibilities in each case.

In assessing the evidence for and against the identity of columbianadin with zosimin it was obvious that only a direct comparison of the two compounds would provide an unequivocal answer. Fortunately, Dr. Nikonov kindly supplied several milligrams of zosimin which enabled such a comparison. Infrared spectra in Nujol mull showed the two to be identical in every respect and, furthermore, no depression of melting point was observed in a mixed melting point determination. The optical rotation of Nikonov's sample of zosimin was $[\alpha]_D^{20}$ + 221.2° (c 1.0, $CHCl_3$). This was substantially lower than that reported by him and is in reasonable agreement with that of columbianadin from two independent laboratories (3, 4). It is, therefore, safe to conclude that columbianadin and zosimin are identical.

[1] More recently, Nikonov (9) has suggested that the difference between zosimin and columbianadin may be in an optically isomeric relationship between columbianetin and zosimol since the 4.05 τ position, in the nuclear magnetic resonance spectrum, of the methine proton found by them in zosimin is contradictory to a tiglate formulation (3).

(1) Nikonov, G. K., and Baranauskaite, D. I., *Khim. Prirodnykh Soedinenii*, **1**, 220(1965).
(2) Nikonov, G. K., and Baranauskaite, D. I., *Chem. Natl. Prod.*, **1**, 169(1966).
(3) Willette, R. E., and Soine, T. O., *J. Pharm. Sci.*, **53**, 275(1964).
(4) Nielsen, B. E., and Lemmich, J., *Acta Chem. Scand.*, **18**, 1379(1964).
(5) Perel'son, M. E., Nikonov, G. K., Pek, G. Yu., and Scheinker, Yu. N., *Dokl. Akad. Nauk SSSR*, **159**, 154(1964).
(6) Mookerjee, A., *Current Sci. India*, **12**, 209(1943).
(7) Soine, T. O., and Jawad, F. H., *J. Pharm. Sci.*, **53**, 990(1964).
(8) Hata, K., and Sano, K., *Tetrahedron Letters*, (No. 14) 1461(1966).
(9) Nikonov, G. K., personal communication, February 20, 1967.
(10) Buckles, R. E., Mock, G. V., and Locatell, L., Jr., *Chem. Rev.*, **55**, 659(1955).

TAITO O. SOINE
KUO-HSIUNG LEE

Department of Medicinal Chemistry
College of Pharmacy
University of Minnesota
Minneapolis, MN 55455

Received November 16, 1966.
Accepted for publication March 1, 1967.

This investigation was supported in whole by grant HE07101 from the National Institutes of Health, U.S. Public Health Service, Bethesda, Md.