meration of viable organisms from petrolatum-based ointments was described and shown to be more efficient than methods now commonly used. An application of the method to the sterility testing of ointments was discussed.

REFERENCES

(1) *Federal Register*, 12, 2223(April 4, 1947), p. 141.8 (c).
(2) Lehrfeld, L., and Donnelly, E. G., *Arch. Opthalmol.*, 40, 39(1948).
(3) Vander Wyk, R. W., and Granston, A. E., THIS JOURNAL, 47, 193(1958).
(4) Bowman, F. W., and Holdowsky, S., *ibid.*, 48, 95(1959).
(5) Holdowsky, S., *Antibiot. Chemotherapy*, 7, 49(1957).
(6) Zobell, C. E., and Conn, J. E., *J. Bacteriol.*, 40, 223(1940).
(7) Stapert, E. M., Sokolski, W. T., and Northam, J. I., *Can. J. Microbiol.*, 8, 809(1962).
(8) Bullock, K., and Booth, N. H., *J. Pharm. Pharmacol.*, 5, 759(1953).
(9) "United States Pharmacopeia," 14th rev., Mack Printing Co., Easton, Pa., p. 87.
(10) *Ibid.*, 16th rev., p. 761.
(11) Marshall, M. S., Gunnison, J. B., and Luxen, M. T., *Proc. Soc. Exptl. Biol. Med.*, 43, 672(1940).
(12) Sokolski, W. T., and Lummis, N. E., *Antibiot. Chemotherapy*, 11, 271(1961).
(13) Sokolski, W. T., unpublished data.
(14) "Deityl Brochure," Givaudan-Delawanna, Inc., New York, N. Y., 1963.

Notes

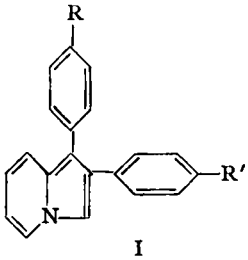
Preparation of *m*- and *p*-Nitrobenzoyl Derivatives of Some Arylindolizines

By VINCENT S. VENTURELLA†

Investigations into the benzoylations of substituted arylindolizines has shown that the reaction is reduced but not prevented by steric or electronic effects.

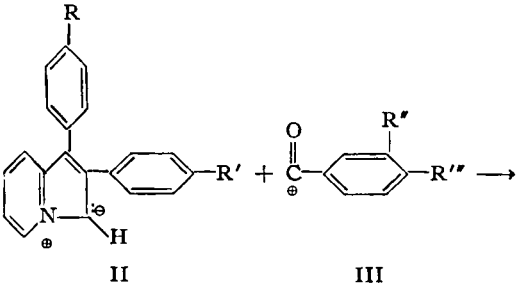
IN A PREVIOUS REPORT (1) it was found that the benzoylation of compounds of structure I proceeded easily and in fair yields except with Compound I *d*. At the time it was indicated that the failure of the reaction to occur with this compound could be attributed to the conjugative and inductive effects of the substituents present in the phenyl rings. Since the benzoylation of the open 1 position is said to occur easily (2), and since the reaction depends upon the contributions from structures II and III, it was desirable to test the effects of the presence of nitro groups on the formation of the benzoylium ion (III) and hence on the formation of a stable benzoyl derivative.

Since hydrolysis of the end product was previously (1) found not appreciable if the reaction was performed in benzene solution below 60° for 1 hour, followed by allowing the mixture to stand 48 hours at room temperature, any change in the formation of the benzoyl derivatives would presumably be because of an increase or decrease in the formation of III.

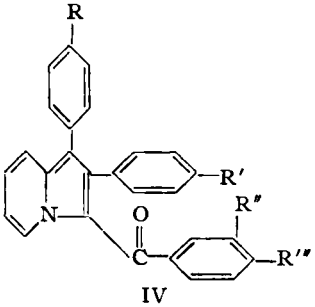


I

I *a*, R = H, R′ = Br
b, R = Cl, R′ = Br
c, R = H, R′ = NO_2
d, R = Cl, R′ = NO_2



II III



IV

IV *a*, R = R″ = H, R′ = Br, R‴ = NO_2
b, R = R‴ = H, R′ = Br, R″ = NO_2
c, R″ = H, R = Cl, R′ = Br, R‴ = NO_2
d, R‴ = H, R = Cl, R′ = Br, R″ = NO_2
e, R = R″ = H, R′ = NO_2, R‴ = NO_2
f, R = R‴ = H, R′ = NO_2, R″ = NO_2

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TABLE I.—SUBSTITUTED BENZOYLINDOLIZINES

Compd.	M.p., °C.(Recrystn. Solv.)	Yield, %	Anal. Calcd. C	H	N	Found C	H	N
IV*a*	220.5 to 222.0, dec. (hot C_6H_6)	66	65.20	3.42	5.63	65.60	3.45	5.34
IV*b*	177 to 178.5 (boiling EtOH)	89	65.20	3.42	5.63	65.55	3.60	5.26
IV*c*	218 to 219.5, dec. (C_6H_6-Petroleum ether)	38	61.01	3.01	5.27	61.14	3.22	5.33
IV*d*	202 to 203.5 (C_6H_6)	64	61.01	3.01	5.27	60.61	3.18	5.48
IV*e*	245.5 to 247.0 (hot C_6H_6)	54	69.99	3.67	9.07	70.29	3.90	9.22
IV*f*	222 to 223.5	46	69.99	3.67	9.07	70.18	3.77	8.78

To test this theory, Compounds I *a*, *b*, and *c* were reacted with *p*- and *m*-nitrobenzoyl chlorides. The *p*-nitro would tend to decrease the formation of III by the conjugative mechanism (the inductive effect being negligible) and the *m*-nitro group would tend to favor the formation of III by a combination of conjugative and inductive effects (3).

These assumptions are followed with Compound I *a*, but fail in the reaction of I *b* with *m*-nitrobenzoyl chloride because the *p*-chlorophenyl substituent present in the 3 position of the indolizine does not favor the formation of II. This effect is also shown to provide a sharp decrease in the formation of IV *c*. A similar anomoly is shown between I *c* and *m*-nitrobenzoyl chloride because the steric repulsion of two proximal nitro groups is unfavorable to the formation of IV. However, the reaction of *p*-nitrobenzoyl chloride with I *c* follows the pattern set by Compound I *a*, *i.e.*, the *p*-nitro group has little effect upon the reactions in this series unless accompanied by a strong tendency to decrease the nucleophilic character of the 1 position of the indolizine nucleus.

Compounds IV *a* through *f* were prepared in the manner previously described (1); the analytical data appear in Table I.

REFERENCES

(1) Venturella, V. S., THIS JOURNAL, **52**, 868(1963).
(2) Holland, D. O., *J. Chem. Soc.*, **1955**, 1504.
(3) Gould, E. S., "Mechanism and Structure in Organic Chemistry," Henry Holt and Co., New York, N. Y., 1959, pp. 218-219.

Quantitative Fluorometric Determination of Panthenol in Multivitamin Preparations

By RENÉ G. PANIER and JEAN A. CLOSE

A new procedure is described for the estimation of panthenol in a multivitamin preparation highly concentrated in sugar. The method involves the quantitative elimination of sugar by crystallization in the presence of ethanol, extraction of panthenol from the dry residue with chloroform, purification of the extract on ion-exchange resins, and final fluorometric determination of β-alanol after alkaline hydrolysis of the effluent. Fluorescence is developed by reaction of β-alanol with ninhydrin and *n*-butanal, for 45 minutes at 60° in the presence of carbonate buffer, pH 9.1. The fluorescent intensity measured at 465 mμ is proportional to the β-alanol concentration in the range between 0.1 and 1 mcg./ml.

DURING THE LAST 10 years, several papers have been published on the quantitative analysis of panthenol in multivitamin pharmaceutical preparations. Chemical methods have been proposed to replace the time consuming microbiological assay. It seems, however, that the problem of the determination of panthenol in highly concentrated sugar preparations has not been solved satisfactorily, and we were recently confronted with that question.

In 1949, Crockaert (1) showed that panthenol and calcium pantothenate could be determined after alkaline hydrolysis as β-alanol and β-alanine, respectively, with the use of 1,2-naphthoquinone-4-sulfonate, while Wollish and Schmall (2) proposed the dosage of pantoyl lactone as ferric hydroxamate, after acid cleaving of the panthenol.

More recently, Zappala and Simpson (3) described a colorimetric method for the determination of panthenol in multivitamin tablets containing 15% sugar. According to these authors, the method of Schmall and Wollish (4) is not applicable to the preparation highly concentrated in sucrose because of the darkening of the solution during the alkaline hydrolysis. They solved the difficulty by extraction of the powdered sample and further purification on ion-exchange resin before estimating β-alanol by chlorination and subsequent iodometry. Their method is unfortunately inapplicable to liquid multivitamin preparations, particularly to syrups with a very high sugar content (more than 50% sucrose).

Our purposes were to determine the panthenol in multivitamin preparations and to perfect a very sensitive routine method capable of answering our control and research problems.

EXPERIMENTAL

Reagents

All the reagents were analytical grade.

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