Colorimetric Assay of Amphotericin B

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AMPHOTERICIN B, like nystatin, is usually assayed by microbiological methods (1, 2). Following the development of the colorimetric assay of nystatin (3), the method was applied to amphotericin B and promising results were obtained.

The procedure of the assay was the same as for nystatin (3), except that the concentration of amphotericin B solution was about 80 mcg./ml. and absorbance measurements were made at 435 mμ. Results of assays of several samples of powder are shown in Table I.

The colorimetric assays show good reproducibility with a standard deviation of about 2%. The results agree well with the microbiological assays which have a standard deviation of about 2.7%.

It was observed that the absorbance of the color extracted into chloroform was lower if the amphotericin B solution was allowed to stand in the presence of sodium hydroxide, indicating that this method, as in the case of nystatin, may also be a stability assay for amphotericin B. Stability experiments and assays of pharmaceutical preparations are now in progress.

TABLE I.—COLORIMETRIC ASSAY OF AMPHOTERICIN B

Sample[a]	Colorimetric Assay[b] (mcg./mg.)	Average (mcg./mg.)	Microbiological Assay[c] (mcg./mg.)
Powder #1	807		
	809		
	772		
	781	792	801
Powder #2	828		
	816		
	820		
	819	821	786
Powder #3	771		
	760		
	765	765	743
Powder #4	877		
	879		
	832	863	864
Powder #5	866		
	886		
	883	878	879
Powder #6	789		
	805	797	802
Powder #7	941		
	920		
	895		
	903	915	908
Powder #8	764		
	796		
	783		
	794		
	804	788	825

[a] Stored at 0° and colorimetric assays performed over a 4-month period. [b] Each value represents an individual assay with an independent sample preparation. [c] Assayed by the turbidimetric method using *Candida tropicalis*.

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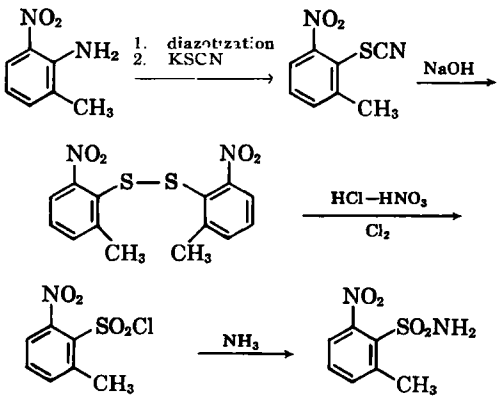
Saccharin Derivatives VII. Synthesis of 7-Nitrosaccharin and 7-Aminosaccharin

By GLENN H. HAMOR

RECENT work has shown that alkyl 4-amino-2-sulfamoylbenzoates possessing marked anticonvulsant activity may be prepared by alcoholysis of 6-nitrosaccharin, followed by reduction (1). Therefore, the preparation of 7-nitrosaccharin was undertaken as a starting material for synthesis of further compounds to be used in a structure-activity correlation study of anticonvulsants. In addition 7-aminosaccharin was synthesized in the hope that it, along with 7-nitrosaccharin, might give useful information concerning the relationship of chemical structure to taste (2).

The parent compound, 6-nitro-*o*-toluenesulfonamide (I), was prepared by the method of Szabo (3) according to the series of reactions shown below.



I

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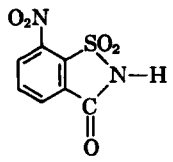
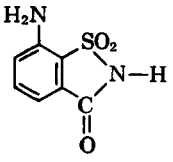
Permanganate oxidation of 6-nitro-*o*-toluenesulfonamide gave 7-nitrosaccharin (II). Reduction of 7-nitrosaccharin by hydrogen with palladium-on-carbon catalyst yielded 7-aminosaccharin (III).



II



III

The 7-nitrosaccharin is essentially tasteless, while 7-aminosaccharin has a sweet taste.

EXPERIMENTAL[1]

7-Nitrosaccharin.[2]—This compound was prepared by alkaline potassium permanganate oxidation of 6-nitro-*o*-toluenesulfonamide by the method used by Noyes (4) to synthesize 6-nitrosaccharin. The 6-nitro-*o*-toluenesulfonamide, m.p. 201–202° [reported m.p. 197–199° (3)], was synthesized according to the series of reactions shown above. Recrystallization of the 7-nitrosaccharin from ethanol gave yields of approx. 25% of yellowish-white crystals, m.p. 262–264° dec. Approx. 30% of unreacted starting sulfonamide was recovered and used in succeeding oxidations.

Anal.[3]—Calcd. for $C_7H_4N_2O_5S$: C, 36.84; H, 1.77. Found: C, 37.02; H, 1.86.

7-Aminosaccharin.—Reduction of 7-nitrosaccharin using hydrogen with palladium-on-carbon catalyst according to standard procedures (5), followed by recrystallization of the product from ethanol gave crystalline 7-aminosaccharin (82%) m.p. 269–270°. Dilute solutions of the compound in ethanol displayed a bluish fluorescence.

Anal.—Calcd. for $C_7H_6N_2O_3S$: C, 42.42; H, 3.12. Found: 42.49; H, 3.09.

[1] Melting points were performed by the capillary tube method and are uncorrected.

[2] *Chem. Abstr.* nomenclature: 7-nitro-1,2-benzisothiazolin-3-one-1,1-dioxide.

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[3] Analyses were performed by Elek Micro Analytical Laboratories, Los Angeles, Calif.

Polytetrafluorethylene Tipped Tablet Punches

By SHELDON SIEGEL†, EDWARD J. HANUS, and JOHN W. CARR

Severe powder sticking to tablet punch surfaces during the production of an effervescent tablet has been overcome by the utilization of punches tipped with polytetrafluorethylene.[1]

The objective of this investigation was to develop an effervescent tablet containing a mixture of sodium isoascorbate and isoascorbic acid which would be rapidly soluble in water and form a solution completely free of turbidity. This tablet was to be used as a source of isoascorbate for meat curing processes to provide a convenient measure for the meat packer who wished to equate his antioxidant requirements to numbers of tablets in preference to units of weight or volume.

The manufacture of an effervescent tablet which yields a clear solution when dissolved in water is not possible when conventional tablet lubricants such as the metallic stearates, mineral oil, etc., are used. For this reason polyethylene glycol 4000 was chosen to act in the dual role of binder and water soluble lubricant. An effervescent tablet blend was prepared and compressed directly on a Stokes model DDS-2 tablet machine into 15-Gm. wafers having a diameter of $1^5/_{16}$ in. The blend had the following composition (1):

Sodium isoascorbate	9.92	Gm./tablet
Isoascorbic acid	2.02	"
Sodium bicarbonate U.S.P. powder	1.06	"
Citric acid anhydrous U.S.P. powder	0.50	"
Polyethylene glycol 4000 (100 mesh)	1.50	"
	15.00	"

When direct compression of this powdered formulation was attempted, severe picking and sticking occurred after compressing relatively few tablets; satisfactory production was impossible because of frequent breakdowns. As an outgrowth of this investigation the utilization of plastics, as components of tablet punches, was studied.

EXPERIMENTAL

Circles of polyethylene film were cut from a plastic bag and bonded to punch faces with a rubber cement. Satisfactory experimental facsimilies of the desired formulation were produced with these punches on a single punch Stokes model F tablet machine. It was noted that the sticking and picking observed in earlier experiments was significantly reduced. Polytetrafluorethylene (2–4) was subsequently investigated because of its inherent self-

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[1] Marketed as Teflon by E. I. duPont de Nemours and Co., Inc., Wilmington, Del.