In mice the LD_{50} was 170 ± 5.15 i.p. 200 ± 21.52 s.c.

Investigated after Zetler's (2) cataleptic method, in i.p. doses of 20 mg./Kg., the compound was found to be a strong antagonist of catalepsy caused by reserpine or chlorpromazine. It has furthermore strongly antagonized the toxicity of reserpine under acute and subacute conditions. Alone it gave rise to slight hyperthermia. In doses of 40 mg./Kg. it has reversed hyperthermia due to reserpine. In rotarod test (3) the compound alone failed to exert any effect in doses of 10–80 mg./Kg. i.p., but doses from 10 mg./Kg. have antagonized incoordination caused by 2.5 mg./Kg. reserpine. In doses of 10 mg./Kg. the compound has intensified the hypertensive effect of epinephrine in cats. After preliminary treatment for 5 days, the effect of epinephrine and norepinephrine on blood pressure has been greatly enhanced. As shown by Knoll's motimeter (4), the compound induced minimal hypermotility when administered in i.p. doses of 20 mg./Kg.

Hypermotility caused by amphetamine has been inhibited in the first 60 min., but in the period from 60–210 min. it has been increased in an extraordinary measure. When administered simultaneously with hexobarbital, the compound has slightly prolonged the narcosis time. However, after premedication over 24 hr., anaesthesia was considerably shortened. The compound has exerted practically no influence on pentylenetetrazol,[1] strychnine, and electroconvulsions.

No analgesic effect could be demonstrated. Monoamino oxidase has not been inhibited either *in vitro* or *in vivo* after premedication for 1 week The compound has shown no noteworthy harmful effect in subacute toxicity tests.

Upon due consideration of the obtained findings, the compound would seem to display an extraordinary likeness in pharmacological properties to tricyclic antidepressants, and as such it may be denoted as a potential antidepressive drug of new chemical type.

A report on detailed pharmacological results is to be published later.

[1] Metrazol, Knoll Pharmaceutical Co., Orange, N.J.

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Reserpine activity—antagonism
Epinephrine hypertensive effect—increased
Amphetamine hypermotility effect—antagonism

Validity of the Doty Reaction as a Stability-Indicating Assay for Isoproterenol in an Aerosol Preparation

Sir:

The sensitivity, accuracy, precision, and manipulative simplicity of the ferro-citrate color reaction described by Doty (1) have led to its wide use for assay of preparations containing isoproterenol, epinephrine, and other catecholamine drugs. The reaction is selective for the catechol function, and it will therefore be stability indicating where the degradation reaction involves the catechol group. Higuchi, Sokoloski, and Schroeter (2) assayed known mixtures of epinephrine and its oxidative degradation products by four procedures; they reported close correspondence between the result obtained by the Doty reaction and the true value, indicating that the reaction is stability indicating where degradation occurs by oxidation. Schroeter, Higuchi, and Schuler (3) described a nonoxidative degradation reaction of epinephrine with bisulfite, used as an antioxidant, where a physiologically inactive substituted benzylsulfonic acid is formed. A similar reaction occurs between bisulfite and isoproterenol (4). The epinephrine-bisulfite reaction product was prepared by the literature method (5). As expected, this product was found to give a color with the Doty reagent, vitiating this assay method for catecholamines in solutions preserved by bisulfite. The Doty method would have obvious limitations in following the stability of those catecholamines subject to racemization in solution (6); however this limitation does not exist with the racemic isoproterenol salts official in the compendia.

Our concern related to the validity of the ferrocitrate assay as a stability method for isoproterenol in a nonaqueous aerosol preparation[1] with thonzonium bromide, ascorbic acid, alcohol, and propellants. In order to ascertain whether or not degradation of isoproterenol in this preparation furnished a product with an intact catechol function, we subjected aged samples to thin-layer chromatography and sprayed the developed chromatograms with Doty reagent prepared according to the NF (7). (The use of this reagent as a selective spray for detection of catechol derivatives on chromatograms has not been described previously. A fuller description of its application will be published elsewhere.)

The aerosol containers were chilled by immersion in a freezing bath of solid carbon dioxide–acetone, opened with pliers, then allowed to warm to room temperature to evolve propellants. The remaining solution was diluted with an equal volume of absolute methanol, and 25-μl. volumes of it and a 600 mcg./ml. solution of reference isoproterenol HCl were spotted 3 cm. from the bottom edge of a commercial precoated 20 × 20 cm. thin-layer plate consisting of a 0.25-mm. thickness of microcrystalline cellulose without binder or phosphor on glass.[2] Before use, the plates were developed in the solvent to about 15 cm. from the starting line and air dried to remove contaminants from the plate. Pretreatment of the plate in this manner eliminated an apparent second front across the chromatogram which resulted in artifactual spots such as those described by Choulis (8). The chromatogram was developed to about 15 cm. from the starting line with a mixture of 2-propanol and 0.1 *N* hydrochloric acid (5:1). After air drying and spraying with the Doty reagent, only one purple spot was obtained at R_f 0.4, corresponding to intact isoproterenol. The detection limit for isoproterenol HCl or epinephrine HCl is below 0.3 mcg. (2% of the amount spotted from the formulation). A volume of 0.5 μl. of 0.6 mg./ml. epinephrine HCl (0.3 mcg.) was added to a sample preparation spot. It was readily detected at R_f 0.2 after chromatographic separation and treatment with Doty reagent. Thus, the method was shown to be capable of separating isoproterenol from a closely related catecholamine and detecting at least 2% of the amount spotted.

The procedure was applied to isoproterenol and thonzonium bromide aerosol samples stored at room temperature for more than 5 years. Examination of stability data obtained for five lots of the formulation during this time revealed no consistent pattern of degradation. Instead, it appears that each sample reacts to the extent of the oxygen present and remains substantially unchanged thereafter. Although some degradation was evident in all of the stability samples, isoproterenol content remained satisfactory throughout the testing period.

[1] Marketed as Nebair by Warner-Chilcott Laboratories, Morris Plains, N. J.

[2] Uniplate coated with Avicel, obtained from Analtech, Inc.

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TLC—analysis, separation

Absolute Stereochemistry and Analgesic Potency of Prodine Enantiomers

Sir:

Stereochemical factors are known to be of importance in analgesic action (1). Among the structures in this well-known class of biologically active compounds, α and β-prodine (Ia and Ib, respectively) (2) distinguish themselves by being highly potent synthetic analgesics possessing two asymmetric centers. While the relative stereochemistries of Ia and Ib are known (3), the absolute configurations and potencies of the optically pure enantiomers have not been reported and are of great interest because they possess an asymmetric center in common with isomethadone (4). In this communication evidence is presented which permits assignment of the complete absolute stereochemistry of these enantiomers.

Treatment of racemic IIa (2) with an equivalent of (+)-tartaric acid in methanol–acetone