and add 100 ml. of water. Attach a reflux water-cooled condensor to the flask and reflux for 1 hour over a Bunsen burner. Cool the solution and filter. Measure the volume of filtrate collected, and if necessary, add water to make 100 ml. This constitutes the litmus solution.

While the above procedure appears lengthy, it requires for the most part only cursory supervision during the various stages of extraction. The entire extraction, both with alcohol and with water, takes place in the same apparatus using only 75 ml. of alcohol.

The resulting solution can be used as such; but if the volume is more than is required for immediate use, it may be freeze-dried and kept until needed. It is feasible to freeze-dry 100 ml. of the solution in the VirTis freeze-dryer. No doubt other types of dryers are also suitable for this purpose.

The yield of dried residue from 100 ml. of extract is 500 ±2 mg. so that reconstitution can be effected by dissolving the powder in water in the proportion of 5 mg. per ml. Dried material so obtained has not been noted to undergo any change on storage for 12 months.

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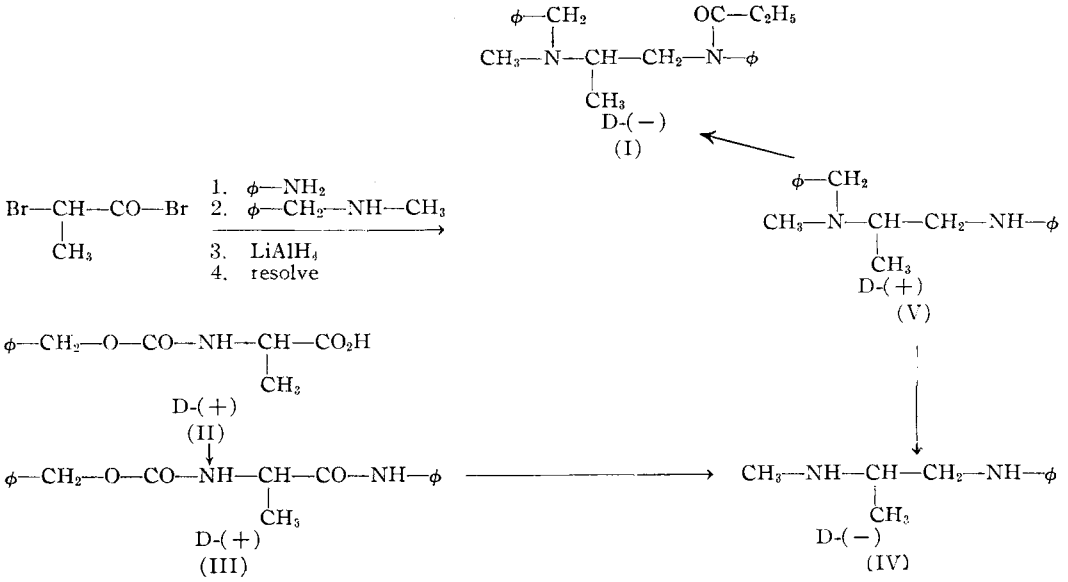
The Absolute Configuration of (-)-N-[2-(Methylbenzylamino)-propyl]-propionanilide

Sir:

The importance of configurational factors on the activity of various synthetic analgesics has been illustrated by several investigators (1–6). These studies have shown that nearly all the activity resides in only one of the enantiomorphs. Beckett (7–10) has determined that the more active enantiomorphs of methadone-type and thiambutene-type analgesics possess the D-configuration. Though there are only a few classes of analgesics of known absolute configuration, a hypothesis has been proposed which attempts to explain this antipodal specificity on the basis of three point contact with a specific receptor in which only one antipode can fit properly (7). The above findings and speculations have prompted us to determine the absolute configuration of (-)-N-[2-(methylbenzylamino)propyl]-propionanilide (I) (11), a member of a new class of potent analgesics (12). We have related the absolute configuration of I to D-alanine by the following sequence



D-Carbobenzoxyalanine (II) (13) was converted to the corresponding anilide (III), m.p. 162–164°, $[\alpha]_D^{30°}$ +28.4° (c 5% in HOAc), by the action of dicyclohexylcarbodiimide.

Anal.—Calcd. for $C_{17}H_{19}N_2O_3$: C, 67.96; H, 6.38; N, 9.32. Found: C, 68.19; H, 6.13; N, 9.66.

Reduction of III with lithium aluminum hydride in tetrahydrofuran afforded the hygroscopic diamine (IV), m.p. 35–36.5°, b.p. 67–71° (0.1 mm.), $[\alpha]_D^{30°}$ −29.2° (c 5% in ethanol). This compound was analyzed as the dipicrate, m.p. 143–144.5°.

Anal.—Calcd. for $C_{22}H_{22}N_8O_{14}$: C, 42.43; H, 3.56; N, 18.00. Found: C, 42.49; H, 3.60; N, 18.23.

The DL-alkylene diamine (V), m.p. 42–44°, was prepared according to the procedure of Wright, Brabander, and Hardy (11, 12). This compound was previously reported as an oil. Optical resolution of V was accomplished *via* its *d*-bitartrate salt. Five recrystallizations from ethanol afforded a bitartrate, m.p. 101–103°, of high purity. Treatment of the bitartrate with aqueous sodium hydroxide regenerated the resolved diamine (V), m.p. 59–61°, $[\alpha]_D^{27°}$ + 31.2° (c 5% in ethanol). The compound formed a monopicrate, m.p. 168–169°.

Anal.—Calcd. for $C_{23}H_{25}N_5O_7$: C, 57.11; H, 5.21; N, 14.48. Found: C, 56.84; H, 5.33; N, 14.38.

Catalytic hydrogenolysis of the resolved (+)-diamine (V) with palladium-on-carbon catalyst at 40 p.s.i. produced diamine IV, $[\alpha]_D^{27°}$ −29.5°, m.p. 34–35°. The infrared spectrum, gas chromatographic retention time, and dipicrate all were identical with those of diamine IV derived from D-alanine.

Treatment of the D-(+)-diamine (V) with propionic anhydride afforded the D-(−)-enantiomer of I, $[\alpha]_D^{27°}$ −45.7° (c 5% in ethanol), b.p. 152–157° (0.3 mm.). This compound exhibited an infrared spectrum and retention time which is identical with racemic I (11). Wright, Brabander, and Hardy (14) have independently prepared the (+)- and (−)-enantiomers of I. Our physical constants are in agreement with their data.

The analgesic potency ratio between the D- and L-forms will be the subject of a future communication.

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Studies on the Mechanism of Biotin Action

Sir:

Biotin has been implicated as an essential factor in a wide variety of carboxylation reactions in biosynthetic pathways, but the exact mechanism by which biotin functions remains unknown. For example, in the biosynthesis of purines, it has been suggested (1) that biotin is required in the carboxylation of 5-aminoimidazole ribotide to 5-aminoimidazole 4-carboxylic acid ribotide, which after several further reactions is converted into inosinic acid. Also, in the preliminary steps of fatty acid synthesis, it has been shown that a biotin-containing enzyme is involved in carboxylation of acetyl CoA to malonyl CoA (2). Studies of fatty acid synthesis in certain cell-free extracts have demonstrated that carbon dioxide is activated by a biotin-containing enzyme and that the activated $CO_2 \sim$ biotin-enzyme complex acts as the carboxylating reagent (3). It is unlikely that the carboxyl group of biotin is transferred in this carboxylation reaction since it is known that biotin is covalently bound to protein, probably through an amide linkage to the ϵ-amino group of lysine (4). We have felt that the most logical manner in which carbon dioxide could be activated by biotin is *via* one of the nitrogens of the imidazolidine ring. Recently, evidence has ac-