trolysis in a cell provided with an aluminum electrode and a platinum spiral, but their results could not be substantiated.

We have successfully applied the rotation aluminum electrode to the amperometric titration of fluoride in aqueous buffers with aluminum nitrate; no good end-point was observed with thorium nitrate as reagent. Hydroxyl ions are the only other ions which anodically depolarize the electrode. They yield anodic limiting currents proportional to concentration. Use of this can be made in the amperometric titration of acids with a strong base. At a *p*H greater than 8 the electrode acts as a *p*OH (*p*H) electrode, but not in acid medium. In acid medium it acts like a *p*F electrode, the potential being somewhat dependent on *p*H in the presence of fluoride. Details concerning this interesting electrode will be reported elsewhere.

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RECEIVED FEBRUARY 2, 1959

INHIBITION OF PYRIDOXAL PHOSPHATE-DEPENDENT ENZYMES BY THE SULFATE ESTERS OF ESTRADIOL, ESTRONE AND DIETHYLSTILBESTROL[1]

Sir:

Systematic studies of kynurenine transaminase in this laboratory have revealed that it is inhibited by extremely low concentrations of estradiol disulfate and diethylstilbestrol disulfate(Table I). Estrone sulfate and pregnanediol glucuronide also inhibited but only at considerably higher levels. Several bile acids inhibited slightly at high concentrations (10^{-3} to 10^{-4} M), probably by denaturation. Unconjugated steroids and related compounds, *e.g.*, estradiol, estrone, diethylstilbestrol, progesterone, pregnanediol, methyl bisdehydrodoisynolic acid and methallenestril were without effect even at saturation levels.

The degree of inhibition varied with the concentration of pyridoxal phosphate, indicating that the estrogen sulfates may compete with the coenzyme for the apotransaminase. Reversibility was shown also by complete disappearance of the inhibitory effect during dialysis. The possibility, suggested here, that other pyridoxal phosphate-dependent enzymes may be inhibited led to tests of the effects of the estrogen sulfates on the activity of muscle phosphorylase (a crystalline preparation) and to the discovery that they inhibit this enzyme also and at very low levels (Table I).

Kynurenine transaminase was purified and assayed as described earlier.[2] Twice crystallized muscle phosphorylase *a* was dissolved in cysteine-glycerophosphate buffer, *p*H 6.8. Aliquots were preincubated at 37° for 30 minutes with the various inhibitors or an equal volume of water, then assayed for activity by the method of Illingworth and Cori.[3]

TABLE I

INHIBITION OF KYNURENINE TRANSAMINASE AND MUSCLE PHOSPHORYLASE BY STEROID CONJUGATES

Inhibitor	Inhibitor concn., M	Per cent. inhibition: Kynurenine transaminase	Per cent. inhibition: Muscle phosphorylase *a*
Estradiol disulfate	2.5×10^{-5}	97	48
	5×10^{-6}	50	19
	5×10^{-7}	9	..
Diethylstilbestrol disulfate	2.5×10^{-5}	99	76
	5×10^{-6}	60	28
	5×10^{-7}	11	..
Estrone sulfate	1×10^{-4}	23	15
	2.5×10^{-5}	9	2
	5×10^{-6}	0	0
Pregnanediol glucuronide	1×10^{-4}	7	..
	2.5×10^{-5}	0	..

Muscle phosphorylase contains tightly-bound pyridoxal phosphate which can be removed to yield preparations that are enzymatically active only in the presence of added pyridoxal phosphate.[4] In the present studies, the reactivation of such preparations with pyridoxal phosphate was inhibited by low concentrations of the estrogen sulfate. The degree of inhibition again was dependent on the concentration of added pyridoxal phosphate.

We believe this to be a unique case of a highly-sensitive *in vitro* estrogen–enzyme interaction in which both diethylstilbestrol and the natural estrogens can participate. From a speculative viewpoint, many of the *in vivo* effects of the estrogens correspond rather well to what we might expect from the demonstrated effects on pyridoxal phosphate-dependent enzymes. As an example, the increased storage of glycogen and protein that occurs in some species under the influence of the estrogens may be regulated through the inhibition of phosphorylase and transaminase enzymes. Experiments designed to test further the relationship of these *in vitro* effects to the physiological actions of the steroid hormones are in progress.

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STEREOCHEMISTRY OF THE DECOMPOSITION OF OPTICALLY ACTIVE N-NITROSO AND N-AMINO α,α'-DIMETHYLIDIBENZYLAMINE

Sir:

Recently it has been shown that the reduction of *cis* or *trans* N-nitroso-2,6-diphenylpiperidine[1] and oxidation of the corresponding N-amino compounds[2] proceeded to give either the *cis* or *trans* diphenylcyclopentane and some olefin. The cyclic compounds were formed with retention of configuration. In order to determine whether the same retention of configuration was operative in linear homologs, optically pure N-nitroso-α,α'-

(1) This work was supported by a grant (A-2294) from the National Institutes of Arthritis and Metabolic Diseases, National Institutes of Health.

(2) M. Mason, *J. Biol. Chem.*, **227**, 61 (1957).

(3) B. Illingworth, and G. T. Cori, "Biochemical Preparations," Vol. 3, John Wiley and Sons, New York, N. Y., 1953, p. 1.

(4) C. F. Cori and B. Illingworth, *Proc. Nat. Acad. Sci.*, **43**, 547 (1957).

(1) C. G. Overberger, J. G. Lombardino and R. G. Hiskey, THIS JOURNAL, **80**, 3009 (1958).

(2) C. G. Overberger, J. G. Lombardino and R. G. Hiskey, *ibid.*, **79**, 6430 (1957).

dimethyldibenzylamine (I) and the corresponding N-amino compound (II) were prepared by the route

$$C_6H_5CH(CH_3)NH_2 + C_6H_5COCH_3 \longrightarrow$$
$$[\alpha]^{25}D\ -40.03^\circ$$
$$C_6H_5CH(CH_3)N{=}C(CH_3)C_6H_5$$
$$III,\ [\alpha]^{25}D\ -97.56^\circ$$

$$III \xrightarrow[H_2]{Pd/C} [C_6H_5CH(CH_3)]_2NH \xleftarrow{HONO}$$
$$IV,\ [\alpha]^{25}D\ -196.3^\circ$$
$$[C_6H_5CH(CH_3)]_2NNO$$
$$I,\ [\alpha]^{20}D\ -259.1^\circ$$

$$I \xrightarrow[EtOH]{Na} [C_6H_5CH(CH_3)]_2N{-}NH_2$$
$$II,\ [\alpha]^{20}D\ -115.8^\circ$$

Catalytic reduction of (III), b.p. 106° (0.2 mm.) (*Anal.* Found: C, 86.12; H, 7.71; N, 6.52) gave 88% of one optical antipode of IV, b.p. 103–105° (0.3 mm.) (*Anal.* Found: C, 85.44; H, 8.36; N, 6.44). Reduction predominantly from one side can be explained by molecular models which show that the benzylidene phenyl cannot rotate freely and is twisted out of the C–C=N plane. This out of plane deformation is supported by spectral evidence. Both the nitrosation of IV to I, m.p. 56–57° (*Anal.* Found: C, 75.55; H, 7.33; N, 10.95) and the reduction of I to II, b.p. 120° (0.1 mm.) (*Anal.* Found: C, 80.14; H, 8.21; N, 11.44) proceeded without racemization. The optical purity of I and II was determined by reconversion to IV, the optical purity of which had been determined previously by catalytic debenzylation to the known α-methylbenzylamine.

Optically pure 2,3-diphenylbutane (V) $[\alpha]^{20}D$ + 85.5° (ethanol), was prepared by nitration and subsequent catalytic reduction of DL-2,3-diphenylbutane to give DL-*p,p'*-diamino-2,3-diphenylbutane (VI) m.p. 84–85.5° (*Anal.* Found: C, 80.08; H, 8.27; N, 11.33) which was resolved using D-tartaric acid. Subsequent deamination gave V.

Hydrosulfite reduction of I proceeded with 90% evolution of nitrogen to give 21.2% *meso* and 43% liquid 2,3-diphenylbutane having an $[\alpha]^{20}D$ of −45.5°. The mercuric oxide oxidation of II proceeded with even less retention of configuration giving 31.2% *meso* and 45% liquid hydrocarbon having an $[\alpha]^{20}D$ −36.4°, the theoretical amount of nitrogen being evolved. The 2,3-diphenylbutanes were the only products identified—no styrene or polystyrene could be isolated.

The stereochemical results for the case reported here suggest that the elimination of nitrogen and formation of coupled product does not proceed by a completely concerted process. In the case of the substituted piperidines[1,2] formation of cyclic product was interpreted as arising from a concerted process while formation of olefinic product was by an unconcerted path. The formation of *meso* product in the linear case reported here and the partially racemized coupled product account for more ionic or free radical character to the transition state or intermediate than for that of the cyclic cases for both the sodium hydrosulfite reduction reaction and the mercuric oxide oxidation. The apparent larger degree of a concerted mechanism for the cyclic cases may be due to geometric factors.[3]

Since the coupled linear product does exhibit some optical activity, it is improbable that the benzyl residues, whether ionic or radical, have an independent existence in excess of time for racemization.

(3) For a comparison of a linear and a cyclic case of similar structure in an azo decomposition see C. G. Overberger, I. Tashlick, M. Bernstein and R. G. Hiskey, *ibid.*, **80**, 6550 (1958). It was shown that radicals formed from a cyclic compound were more difficult to trap than linear ones of similar structure.

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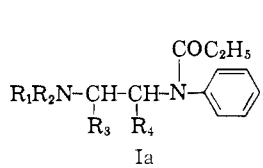
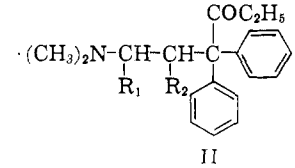
N-(*tert*-AMINOALKYL)-PROPIONANILIDES: A NEW SERIES OF POTENT ANALGESICS

Sir:

We wish to report the establishment of a new series of potent analgesics of novel chemical structure (Ia). The compounds were designed as analogs of methadone (IIa) and isomethadone (IIb) and retain the steric requirements of potent



Ia



II

Ib, $R_1 = C_6H_5(CH_2)_2$, $R_2 = R_3 = CH_3$, $R_4 = H$
Ic, $R_1R_2 = (CH_2)_5$, $R_3 = H$, $R_4 = CH_3$
IIa, $R_1 = CH_3$, $R_2 = H$
IIb, $R_1 = H$, $R_2 = CH_3$

analgesics as set forth by Beckett and Casy and others.[1]

Screening for analgesic activity was accomplished by a sequential modification[2] of the mouse hot plate method of Woolfe and Macdonald.[3] Evaluation of the more active compounds by a modification of the rat tail radiant heat procedure of D'Amour and Smith,[4] and subsequent toxicity studies, led to the selection of N-[2-(methylphenethylamino)-propyl]-propionanilide (Ib) sulfate, *diampromid*, and N-(1-methyl-2-piperidinoethyl)-propionanilide (Ic) hydrochloride, *phenampromid*, as candidates for trial in man. Preliminary clinical results indicate that the potency of diampromid lies between that of meperidine and morphine and the potency of phenampromid approximates that of meperidine.

A benzene solution of methylphenethylamine[5] and 2-bromopropionanilide[6] was heated under re-

(1) (a) A. H. Beckett and A. F. Casy, *J. Pharm. and Pharmacol.*, **6**, 986 (1954); (b) O. J. Braenden, N. B. Eddy and H. Halbach, *Bull. World Health Organization*, **13**, 937 (1955).

(2) A. C. Osterberg, J. D. Haynes and C. E. Rauh, *J. Pharm. Acad. Exptl. Therap.*, **122**, 59A (1958). Details of the screening and subsequent pharmacological studies will be reported elsewhere by A. C. Osterberg, *et al.*

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(4) F. E. D'Amour and D. L. Smith, *ibid.*, **72**, 74 (1941).

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(6) A. Tigerstedt, *Ber.*, **25**, 2919 (1892).