

Preparation of *meso*-, (\pm)-, and Optically Active Forms of 3,6-Bis-(1-hydroxy-1-phenylpropyl)- and 3,6-Bis-(1-hydroxy-1-phenylethyl)-1,2,4,5-tetrazines *via* a New Synthesis of 1,2-Dihydro-1,2,4,5-tetrazines from Amidines

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The amidinium chlorides (IVa and b) were converted by the action of hydrazine hydrate into mixtures of the *meso*- and (\pm)-dihydrotetrazines (IIa and b), identical with those obtained from the more usual route *via* the imidate salts (IIIa and b). The dihydrotetrazines (IIa and b) were readily oxidised to the tetrazines (Ia and b). Fractional crystallisation of the tetrazines (Ia and b) or their dihydro-derivatives yielded pure samples of the *meso*- and (\pm)-compounds (Ia and b; IIa and b). The use of optically active amidinium chlorides (IVa and b) led to the formation of the optically active dihydrotetrazines (IIa and b) and tetrazines (Ia and b) by subsequent oxidation. Admixture of the (+)- and (-)-forms of compound (Ib) proved the (\pm)-tetrazine to be the lower-melting diastereoisomer. Absolute configurations and o.r.d. results for compounds (I) and (II) are also discussed.

3,6-DISUBSTITUTED 1,2,4,5-TETRAZINES(I) are usually prepared *via* the corresponding, readily oxidisable dihydro-1,2,4,5-tetrazines (II) which can be obtained by the action of hydrazine on imidate hydrochlorides (III).¹⁻⁴ We thought that amidines (IV) would also react with hydrazine to give dihydrotetrazines and that this route would make possible the preparation of optically active tetrazines from suitable precursors. Roger and Neilson^{5,6} have prepared the imidate salts (IIIa and b; R² = Et)

and the corresponding amidinium chlorides (IVa and b). These they resolved by formation of salts with the mandelic acids.^{5,6} The amidines (IVa and b) were studied because they would not be subject to racemisation under the strongly basic conditions of the tetrazine synthesis.

The optically inactive 3,6-bis-(1-hydroxy-1-phenylethyl)- and 3,6-bis-(1-hydroxy-1-phenylpropyl)-1,2-dihydro-1,2,4,5-tetrazines (IIa and b) were prepared by treatment of the imidate hydrochlorides (IIIa and b)

¹ A. Pinner, *Ber.*, 1893, **26**, 2126; 1894, **27**, 984.

² V. P. Wystrach, 'Heterocyclic Compounds,' vol. 8, ed. R. C. Elderfield, Wiley, New York, 1967, p. 105.

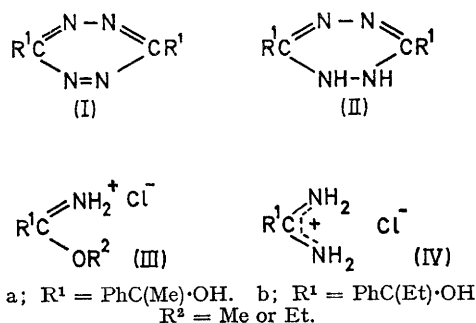
³ K. M. Watson, 'Enciclopedia Internazionale Di Chimica,' Edizioni Pem, Rome, in the press.

⁴ P. Yates, O. Meresz, and H. Morrison, *Tetrahedron Letters*, 1967, **1**, 77 (see also p. 1575).

⁵ R. Roger and D. G. Neilson, *J. Chem. Soc.*, 1959, 688.

⁶ R. Roger and D. G. Neilson, *J. Chem. Soc.*, 1961, 3181.

with hydrazine hydrate in ethanol. The dihydro-compounds were then oxidised by nitrous acid to the corresponding tetrazines (Ia and b). The inactive dihydrotetrazines (IIa and b) were also prepared by the action of hydrazine hydrate in warm ethanol on the



(±)-amidinium chlorides (IVa and b). This new route permitted the synthesis of (–)-dihydrotetrazine (IIa) and the (+)- and (–)-compounds (IIb). This reaction was cleaner than that starting from the imidate and the yields were satisfactory.

All preparations of the optically inactive compounds (I) and (II) gave mixtures of the *meso*- and (±)-forms. This made purification difficult and reduced the yield of the pure diastereoisomeric products. In particular, separation by fractional crystallisation of the lower melting forms of (Ia) and (IIa) was difficult. Moreover, recrystallisation of the dihydrotetrazines in solvents such as alcohol or acetone invariably led to some oxidation, presumably by dissolved oxygen, although the use of benzene and cyclohexane was more satisfactory. Because of this oxidation it was sometimes more advantageous to obtain pure isomers by recrystallisation of the tetrazines rather than the dihydro-compounds, and then to reduce these back to the dihydro-tetrazines with, *e.g.*, sodium dithionite.

Stern, English, and Cassidy⁷ have put forward evidence that for centrosymmetric molecules, the *meso*-form usually, but *not* always, has a higher m.p. than the (±)-form, and on this basis configurations have been allocated to tetrazine diastereoisomers.⁸ We have, however, confirmed that this hypothesis does apply to compounds (Ib) and (IIb), as equimolar samples of (+)- and (–)-(Ib), when mixed and recrystallised from aqueous ethanol, gave a product with m.p. and i.r. spectrum identical with those of the lower-melting, more soluble isomer of (Ib) obtained by fractional crystallisation. In view of the close relationship between the compounds, it is assumed that the lower-melting isomers of (Ia) and (IIa) are likewise the (±)-forms.

In both cases, the (*R*)-(–)-amidinium chlorides (IVa and b) yielded the (+)-dihydrotetrazines (IIa and b) and subsequently the (+)-tetrazines (Ia and b) by oxidation.

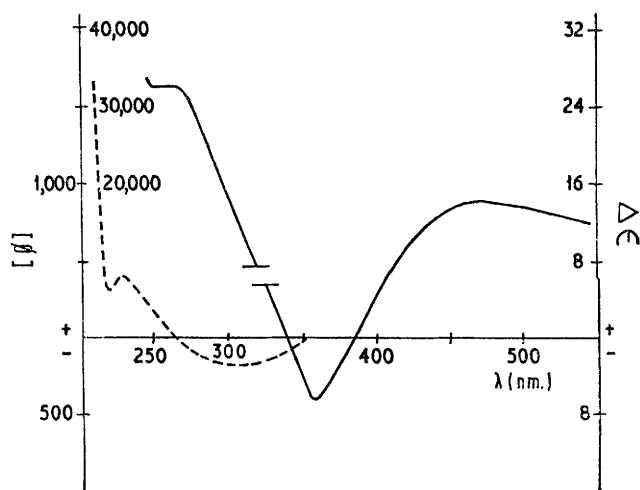
⁷ R. Stern, J. English, jun., and H. G. Cassidy, *J. Amer. Chem. Soc.*, 1957, **79**, 5797.

⁸ R. A. Carboni and R. V. Lindsey, jun., *J. Amer. Chem. Soc.*, 1958, **80**, 5793.

These (+)-compounds (I and II) must, therefore, belong to the *R,R*-series.

The u.v. spectrum of a dihydrotetrazine (in methanol) shows a broad band in the region of 300 nm., and a more intense band centred around 225 nm. The c.d. curve of (+)-dihydrotetrazine (IIb) shows a trough at 300 nm. and a peak at 228 nm. (see Figure). This suggests that the o.r.d. curve of (+)-dihydrotetrazine (IIb) should be interpreted as a Cotton effect of negative sign (minimum at 356 nm., $a -331$) imposed on an intense positive background. The turning point recorded at 455 nm. is thus not a true extremum but arises because of the superimposition of the positive and negative Cotton effects. A similar type of behaviour has been recorded for (–)-ephedrine.⁹

The results for the tetrazines are best illustrated by reference to compound (Ia), which could be examined over a wide wavelength range. This (+)-tetrazine exhibits a c.d. curve in methanol with weak, double negative maxima (560 and 520 nm.) associated with the



O.r.d. (—) and c.d. (---) curves of (+)-dihydrotetrazine (IIb)

$n \rightarrow \pi^*$ nature of this transition.² The o.r.d. of (+)-(Ia) shows a trough at 599 nm. and an inflection at 485 nm. ($a -6$). This is again superimposed on an intense positive background which gives rise to a Cotton effect ($a +293$) in the range 279–218 nm. The results for (+)-(Ib) are similar but the low wavelength extremum was not reached. (Despite the similarity in results, the tetrazines and dihydrotetrazines must be considered separately, as the chromophores are different.)

EXPERIMENTAL

All rotations were measured at 5461 Å.

3,6-Bis-(1-hydroxy-1-phenylpropyl)-1,2,4,5-tetrazines (Ib). —Optically inactive forms. (a) From amidinium chloride (IVb). The (±)-amidinium chloride (IVb) (2.1 g.) in ethanol (20 ml.) and hydrazine hydrate (99%; 2.5 ml.) were heated under nitrogen at 60° for 3 hr. The solution

⁹ I. P. Dirkx and T. J. De Boer, *Rec. Trav. chim.*, 1964, **83**, 535.

was then poured into water and set aside. The resultant white precipitate of impure dihydrotetrazine (IIb) was dissolved in ethanol and stirred with sodium nitrite (4.7 g.) at 50–60° for *ca.* 15 min.¹⁰ Sulphuric acid (m; 35 ml.) was added dropwise and heating was continued for a further 15 min. The addition of acid and heating was repeated, and the solution was then poured into ice-water. Repeated recrystallisation of the resultant tetrazine from aqueous ethanol gave a number of crops of different m.p.s. These included the *meso*-tetrazine (Ib) as crimson needles, m.p. 161–162° (Found: C, 68.4; H, 6.7; N, 15.8. $C_{20}H_{22}N_4O_2$ requires C, 68.6; H, 6.3; N, 16.0%), and the (±)-tetrazine (Ib) as crimson plates, m.p. 101.5–102° (Found: C, 68.8; H, 6.3%). The gross purified yield was 32%.

(b) *From imidate hydrochloride.* (±)-Imidate hydrochloride (IIIb; $R^2 = Me$) (2.3 g.) in ethanol (7 ml.) and hydrazine hydrate (99%; 2.5 ml.) were left together, overnight, and the resultant dihydrotetrazine (IIb) was filtered off. More product precipitated when the filtrate was added to water. The dihydrotetrazine was oxidised as before, yielding tetrazine (Ib). The m.p.s of the purified isomers agreed with those already quoted above; yield 22%.

Optically active forms. (+)-Amidinium chloride (IVb) (1 g.), $[\alpha] +50.0^\circ$ (in water) (lit.,⁸ $[\alpha] \pm 49.0^\circ$), similarly gave (–)-3,6-bis-(1-hydroxy-1-phenylpropyl)-1,2,4,5-tetrazine (0.6 g.), m.p. 109–110°, as crimson needles from aqueous ethanol (Found: C, 68.6; H, 6.3%). (–)-Amidinium chloride (IVb), $[\alpha] -47.5^\circ$ (in water), similarly gave (+)-dihydrotetrazine (IIb) which was purified then oxidised to the (+)-tetrazine, m.p. 110–110.5°. The i.r. spectra of the optical isomers were identical. The intense colour of these compounds precluded the use of a visual polarimeter to find the specific rotations.

(±)-Tetrazine (Ib) by *Admixture.*—Equal weights of the (+)- and (–)-tetrazines (Ib), when mixed and recrystallised from aqueous ethanol, gave the (±)-tetrazine (Ib) as plates, m.p. 101.5–102°. This compound showed no mixed m.p. depression with the lower-melting sample obtained by synthesis and fractional crystallisation. The i.r. spectra were also identical.

3,6-Bis-(1-hydroxy-1-phenylpropyl)-1,2-dihydro-1,2,4,5-tetrazines (IIb).—*Optically active forms.* Solid sodium dithionite¹¹ was added to the (–)-tetrazine (0.2 g.) (Ib) in aqueous ethanol (1:1; 6 ml.) and the mixture was shaken until the red colour had disappeared. It was then poured into water (50 ml.) and the resultant precipitate yielded the (–)-dihydrotetrazine (IIb), $[\alpha] -227.4^\circ$ (*c* 0.14, *l* 2, in MeOH), as needles (52%), m.p. 117.5–118.5° (from cyclohexane) (Found: C, 67.8; H, 6.8; N, 16.0. $C_{20}H_{24}N_4O_2$ requires C, 68.2; H, 6.8; N, 15.9%).

(–)-Amidinium chloride (IVb) (0.63 g.), $[\alpha] -47.5^\circ$ (in water) (lit.,⁸ $[\alpha] \pm 49.0^\circ$) gave, on treatment with hydrazine hydrate as already described, crude (+)-dihydrotetrazine * (IIb) (0.37 g.) which, after recrystallisation from cyclohexane, had m.p. 118–119°, $[\alpha]_D +217.3^\circ$ (*c* 0.56, *l* 2, in MeOH). The purified compound (0.24 g.) had an i.r. spectrum identical with that of the (–)-isomer.

Optically inactive forms (IIb). (±)- and *meso*-Tetrazines (Ib) were reduced with dithionite, as described for the (–)-isomer, to give (±)-dihydrotetrazine * (IIb), m.p. 107.5–108.5°, and *meso*-dihydrotetrazine (IIb), m.p. 159–

160° (from cyclohexane) (in the case of the *meso*-compound, the reduction was slower but was facilitated by warming) (Found: C, 68.5; H, 6.7%).

3,6-Bis-(1-hydroxy-1-phenylethyl)-1,2-dihydro-1,2,4,5-tetrazines (IIa).—*Optically inactive forms.* α -Phenyl-lactimidate hydrochloride (IIIa) and α -phenyl-lactamidinium chloride (IVa) were converted by the action of hydrazine hydrate, as already described, into mixtures of the (±)- and *meso*-dihydrotetrazines (IIa) in 52 and 60% yield respectively. Repeated crystallisation of this mixture from benzene gave an isomer, presumed to be the *meso*-dihydrotetrazine, as plates, m.p. 180–182° (Found: C, 66.9; H, 6.3; N, 17.3. $C_{18}H_{20}N_4O_2$ requires C, 66.7; H, 6.2; N, 17.2%).

The lower-melting isomer was difficult to obtain but appeared to have m.p. 154–155° [presumed to be the (±)-compound]. Fractional crystallisation of the tetrazine (Ia) (see later) and subsequent reduction with dithionite followed by extraction with ether was a more satisfactory route to this isomer * (m.p. 154–155°).

Optically active form. (+)- α -Phenyl-lactamidinium chloride, $[\alpha] +46.2^\circ$ (in water) (lit.,⁵ $+54.8^\circ$), similarly gave (–)-dihydrotetrazine * (IIa) as needles (29%), m.p. 122–123° (from benzene), $[\alpha] -165.0^\circ$ (*c* 0.2, *l* 2, in MeOH).

3,6-Bis-(1-hydroxy-1-phenylethyl)-1,2,4,5-tetrazines (Ia).—*Optically inactive forms.* Oxidation of the high-melting *meso*-dihydrotetrazine (IIa) with sodium nitrite-sulphuric acid as already described yielded *meso*-tetrazine (Ia) as vermilion plates (90%) (from aqueous ethanol), m.p. 186–187° (Found: C, 66.7; H, 5.7; N, 17.6. $C_{18}H_{18}N_4O_2$ requires C, 67.1; H, 5.6; N, 17.4%).

Similarly, oxidation of the crude dihydrotetrazine (IIa) (mixture of unseparated isomers) gave vermilion powders (70%) with indefinite m.p. Repeated recrystallisation from aqueous ethanol of this crude tetrazine yielded the *meso*-tetrazine (Ia), identical with that just described, and also a microcrystalline vermilion powder, the presumed (±)-tetrazine (Ia), m.p. 133–134° (from aqueous ethanol) (Found: C, 67.3; H, 6.0%).

(–)-Tetrazine (Ia).—Oxidation of (–)-dihydrotetrazine (IIa), $[\alpha] -165.0^\circ$ (in MeOH) in the usual way gave (–)-tetrazine (Ia) as vermilion needles, m.p. 121–121.5° (from aqueous ethanol) (Found: C, 67.4; H, 5.5%). The intense colour precluded the use of a manual polarimeter to determine the specific rotation.

O.r.d. Measurements.—(+)-Tetrazine (Ia). Run as the enantiomer (E): $[\phi]_{645} +170^\circ$, $[\phi]_{589} +60^\circ$ tr, $[\phi]_{485} +670^\circ$ infl, $[\phi]_{279} +11,500^\circ$ pk, $[\phi]_{265} 0^\circ$, $[\phi]_{218} -17,800^\circ$ tr, $[\phi]_{213} 0^\circ$!

(+)-Tetrazine (Ib). $[\phi]_{645} +120^\circ$, $[\phi]_{602} +20^\circ$ tr, $[\phi]_{485} +695^\circ$ infl, $[\phi]_{290} +7190^\circ$ pk, $[\phi]_{278} 0^\circ$, $[\phi]_{240} -18,580^\circ$!

(+)-Dihydrotetrazine (IIa). $[\phi]_{345} -1120^\circ$ tr, $[\phi]_{330} 0^\circ$, $[\phi]_{208} +67,000^\circ$ pk, $[\phi]_{204} +64,000^\circ$! (E).

(+)-Dihydrotetrazine (IIb). $[\phi]_{546} +723^\circ$, $[\phi]_{455} +883^\circ$ (turning point), $[\phi]_{383} 0^\circ$, $[\phi]_{356} -448^\circ$ tr, $[\phi]_{344} 0^\circ$, $[\phi]_{263-258} +32,620^\circ$ infl, $[\phi]_{244} +32,870^\circ$ pk?!

C.d. Measurements.—(+)-Tetrazine (Ia) (E). $\Delta\epsilon_{560} -8.4 \times 10^{-2}$, $\Delta\epsilon_{520} -12.8 \times 10^{-2}$. There was also some suggestion of negative maxima around 750 nm. but these were too close to experimental error to be reliable.

(+)-Dihydrotetrazine (IIb). $\Delta\epsilon_{300} -3.06$, $\Delta\epsilon_{228} +6.48$, $\Delta\epsilon_{208} +26.6$.

Methanol was used as solvent for all the o.r.d. and c.d. measurements. The o.r.d. curves were run with a

¹⁰ R. Huisgen, J. Sauer, and M. Seidel, *Annalen*, 1962, **654**, 146.

¹¹ P. Truitt and L. T. Creagh, *J. Org. Chem.*, 1963, **28**, 1910.

* In all cases, spot oxidation of the dihydrotetrazines gave the intense red colouration indicative of the presence of tetrazine.

Bellingham and Stanley/Bendix Ericsson automatic recording spectropolarimeter, Polarmatic 62 within the temperature range 20—25° and at concentrations of 0.2—2 mg./ml.

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c.d. curve of compound (IIb), also Dr. B. Sjöberg, Astra, Södertälje, Sweden, for the c.d. curve of compound (Ia). We also thank Dr. M. Scopes, Westfield College, London, for discussion on the nature of the o.r.d. curves and Mr. M. J. Band and Mrs. E. Morton for technical assistance.

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