

CHARACTERISATION OF L-DOPA IN SEEDS OF *MUCUNA SLOANEI*

L-DOPA [3-(3, 4-dihydroxyphenyl) alanine] is being used for symptomatic relief of Parkinson's disease¹. *Mucuna* species (*M. puriens*, *M. andreana*, *M. mutiana*, *M. boltoni*, *M. erens*, *M. sloanei*, *M. purita*, etc.) are considered to be the richest natural sources of free L-dopa². It is mainly concentrated in the seed embryo of these plants in concentrations of 6-9% w/w². There has been no report so far on the L-dopa content of *M. sloanei* grown in Nigeria. Quickening interest in the use of L-dopa in the treatment of Parkinsonism leads us to investigate and characterise this compound in *Mucuna sloanei*, a plant which is grown widely in deciduous, secondary and Savannah regions of the world, especially Nigeria, Ghana and Sierra Leone.

Experimental

Finely ground, defatted seed (50 g) was heated on a steam bath with 50 ml of 0.1N HCl for 5 minutes. After cooling, the mixture was shaken vigorously with 30 ml of 95% ethanol, and centrifuged. The supernatant was separated and an additional 50 ml of 95% ethanol was added to the residue and shaken vigorously to dissolve any L-dopa remaining. The sample was again centrifuged and the alcohol was mixed with the original supernatant and the residue discarded. The alcohol layer was subjected to T.L.C. analysis on silica gel G (0.25 mm) using solvent system propanol, ethyl acetate, water and acetic acid (20:19:10:1). A 0.1% solution of pure L-dopa in 0.1N HCl was used as a reference substance. Ninhydrin reagent was used for the detection of L-dopa on the chromatogram. A grey-blue colour was obtained for L-dopa in seed extract with R_f 0.60, a value identical with the reference L-dopa on the same plate.

Further confirmation of the identity and configuration of L-dopa was obtained by infra-red spectrum employing Perkin-Elmer instrument. IR spectrum of L-dopa isolated from the seed was in good agreement with that of pure L-dopa.

Estimation of L-dopa in seeds of *M. sloanei* was carried out by a colorimetric method described earlier¹ and was found to be 3.34% w/w dry powder. This suggests that Nigerian variety of *M. sloanei* has a low yield of L-dopa compared to the value of 6-9% for the same species grown in U.S.A.².

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SYNTHESES OF 5, 5'-(*p*-PHENYLENEDIAMINE-DISAZO)-, AND 5, 5'-(*o*-DIANISIDINEDISAZO)-8-HYDROXYQUINOLINE

THE formation of a disazo dye of 8-hydroxyquinoline by coupling with benzidine is well known¹. Here, a pyridine solution of 8-hydroxyquinoline has been used for preparing disazo dyes with *p*-phenylenediamine and *o*-dianisidine. Pyridine medium is very suitable for coupling reactions^{2,3}.

Experimental

Preparation of 5, 5'-(p-phenylenediaminedisazo) and 5, 5'-(o-dianisidinedisazo)-8-hydroxyquinoline.—0.1 Mole of (i) *p*-phenylenediamine dissolved in 300 ml hot water containing 20 ml of concentrated HCl, or (ii) *o*-dianisidine dissolved in 30 ml of concentrated HCl and 400 ml of boiling water, is cooled to 5° with ice, 30 ml of concentrated HCl added, and the solution tetraazotised with 0.2 mole of a concentrated aqueous sodium nitrite solution. After a brisk effervescence of gas, the solution becomes clear and gives only a faint blue colour with starch-iodide paper. This solution is poured slowly into a solution of 0.2 mole of 8-hydroxyquinoline in 50 ml of dry pyridine and the mixture is stirred for ca 1 h and is kept overnight. The solid is filtered, washed with water and dried at 150°.

The purity of products was established by analyses and TLC.

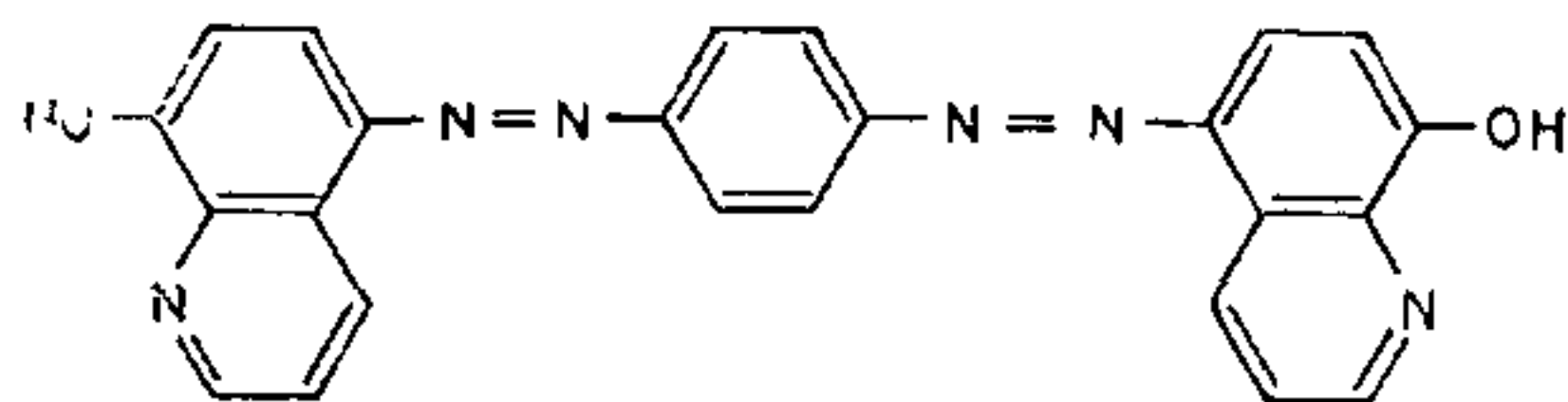
Both the compounds are dark red brown in colour, amorphous, sparingly soluble in chloroform, carbon-tetrachloride, ethanol, etc., but fairly soluble in dimethylformamide. Melting points (uncorrected) of 5, 5'-(*o*-dianisidinedisazo)-8-hydroxyquinoline and 5, 5'-(*p*-phenylenediaminedisazo)-8-hydroxyquinoline are respectively 265° and 200° (d). These compounds behave as excellent ligands and form metal complexes which are useful for analysis.

Elemental analysis indicate the formula $C_{24}H_{18}N_6O_2$ for 5, 5'-(*p*-phenylenediaminedisazo)-8-hydroxyquinoline (found C=67.89%, H=4.45%, N=19.23%; required C=68.23%, H=4.30%, N=19.96%). For 5, 5'-(*o*-dianisidinedisazo)-8-hydroxyquinoline the formula is $C_{32}H_{24}N_6O_4$ (found C=68.52%, H=4.42%, N=14.97%; required C=69.05%, H=4.35%, N=15.10%).

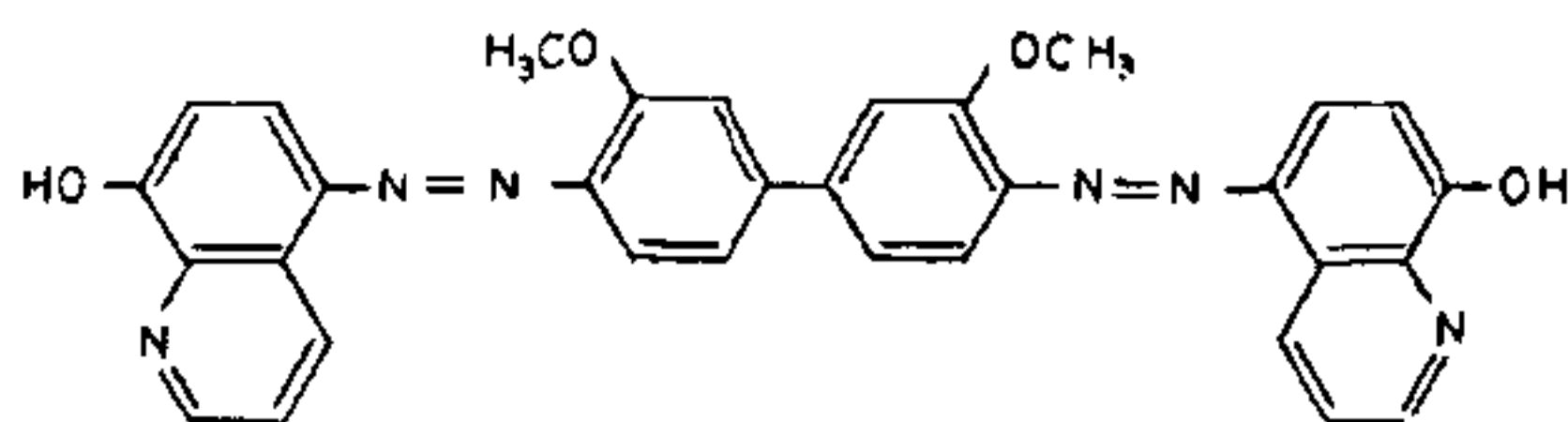
The i.r. spectra (in Nujol/mull) shows bands at 3100, 1570, and 1375 cm^{-1} corresponding to O-H stretching, N=N stretching and C-N vibrations⁴ respectively in 5, 5'-(*p*-phenylenediaminedisazo)-8-hydroxyquinoline

In 5, 5'-(*o*-dianisidinedisazo)-8-hydroxyquinoline, bands are observed at 3105, 1572 and 1370 cm^{-1} , corresponding to the above three assignments, as well as at 1220, 1020, 430 cm^{-1} due to OCH_3 .

On the basis of structure proposed by Boyd¹ for the disazo dye derived from 8-hydroxyquinoline and benzidine, the structures of the dyes reported here are



I. 5, 5'-(*p*-phenylenediaminedisazo)-8-hydroxyquinoline.



II. 5, 5'-(*o*-dianisidinedisazo)-8-hydroxyquinoline.

The coupling occurs on position five, *i.e.*, *p*- to the OH group of 8-hydroxyquinoline and not at position seven *i.e.*, *o*- to the OH group⁵. This is evident because though the *o*- and *p*-positions are sites of high electron density, *p*- is favoured^{6,7} because of the following factors: (i) Steric effect due to large entering group⁸ (tetrazonium electrophile), prevents coupling at position seven. (ii) Polarisability effect (+E) created by the electrophile selectively activates *p*-position⁹. (iii) Inductive effect in the phenoxide ion (−I) through its direct effect create an electrostatic potential difference between *o*- and *p*-positions making *p*- more rich in electron density¹⁰. The above two effects result in the establishment of considerable electron density difference, and the tetrazonium cation which is very sensitive to small electron density differences¹¹ due to its lower stability¹², concentrates at the *p*-position. (iv) Furthermore mesomeric effect in the phenoxide ion can give both *o*- and *p*- quinonoid transition state for *o*- and *p*-substitution respectively but since *p*-quinonoid form is much more stable¹³ it tends to couple at terminal (*p*-) carbon atom in the conjugated series of double bonds.

Work on the metal complexes of these dyes is in progress and will be reported in subsequent communications.

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NEW METHODS FOR THE SYNTHESIS OF BENZOXAZOLIN-2-ONES

Two new synthetic methods have been developed to give good yields of benzoxazolin-2-ones (I).

Benzoxazolin-2-ones (I) are biologically versatile compounds¹⁻⁵. The reaction of phosgene⁶ with *o*-aminophenols or their fusion with urea⁷ are the general methods for obtaining them; while these methods give good yields of unsubstituted benzoxazolin-2-one, the yields are generally lower in the case of substituted derivatives. In addition, the use of phosgene is hazardous. We wish to report two new methods for the synthesis of I.

In the first procedure (A) *o*-aminophenol (0.1 mole) and urea (0.11 mole) were refluxed with excess of dry pyridine for about 14 hrs. Working up the reaction mixture furnished I (R = H) as a crystalline solid, m.p. 136–138°^{6,7} in 80% yield. The method was successfully applied for the preparation of substituted I (R = CO₂H, CO₂Me) with excellent results.