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SHORT COMMUNICATION

MINOR INDOLE ALKALOIDS OF *ALSTONIA MACROPHYLLA*

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Key Word Index—*Alstonia macrophylla*; Apocynaceae; alkaloids; affinisine; picrinine; picralstonine.

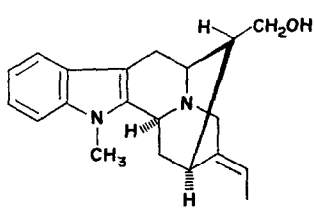
Abstract—Three indole alkaloids have been isolated from *Alstonia macrophylla* Wall. Two of these have been identified as affinisine (I) and picrinine (II). The third, a new base designated *picralstonine*, has been shown to possess structure (III).

INTRODUCTION

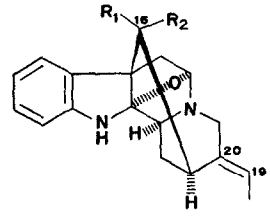
A NUMBER of monomeric and dimeric indole alkaloids have been isolated from *Alstonia macrophylla* Wall. (Fam. *Apocynaceae*).[1-3] Further chemical examination of the minor alkaloids obtained from the ethanolic extract of the leaves of this plant has resulted in the isolation of three more basic constituents, the characterization of which is described in the present communication.

RESULTS

The first alkaloid (0·003% yield), $C_{20}H_{24}ON_2$ (M^+ 308), m.p. 185°, $[\alpha]_D^{25}$ +27° ($CHCl_3$) was identified as affinisine (I)[4] by comparison with an authentic sample (MS, m.m.p., co-TLC and superimposable IR spectra).



(I)



(II) $R_1 = H$, $R_2 = CO_2CH_3$
(III) $R_1 = CO_2CH_3$, $R_2 = H$

The second base (0·005% yield), $C_{20}H_{22}O_3N_2$ (M^+ 338), m.p. 223–224°, $[\alpha]_D^{25}$ −44° ($CHCl_3$) was shown to be identical with picrinine (II)[5-7] from MS, m.m.p., co-TLC and superimposable IR spectra with an authentic sample.

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[2] M. HESSE, *Indolalkaloide in Tabellen, Ergänzungswerk*, Springer, Berlin (1968).
[3] A. CHATTERJEE, *Alkaloids of Alstonia macrophylla Wall.* Published on the occasion of the seventieth birthday of Prof. T. R. Seshadri, Prentice-Hall, Delhi (1970).
[4] M. P. CAVA, S. K. TALAPATRA, J. A. WEISBACH, B. DOUGLAS, R. F. RAFFAUF and O. RIBEIRO, *Chem. & Ind.* 1193 (1964).
[5] A. CHATTERJEE, B. MUKHERJEE, A. B. RAY and B. DAS, *Tetrahedron Letters* 3633 (1965).
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[7] B. MUKHERJEE, D. Phil. Thesis, Calcutta University (1967).

The third alkaloid, a new compound designated *picralstonine*, was isolated in 0·001% yield from 1·5 kg of the leaves of *A. macrophylla*. Due to the extreme paucity of material only a limited number of spectral studies and reactions could be carried out with this compound. Picralstonine, m.p. 200°, $[\alpha]_D^{25}$ −90° ($CHCl_3$), $C_{20}H_{22}O_3N_2$ (M^+ 338·162332; required for $C_{20}H_{22}O_3N_2$: 338·163032) exhibited the colour reactions (HNO_3—instantaneous green changing to yellow; ceric ammonium sulfate in phosphoric acid—dirty orange changing slowly to greyish-green) similar to those of picrinine (II) and its congeners.[7] The UV spectrum of the base (λ_{max}^{EtOH} 234, 286 nm; log ϵ: 3·81, 3·40 respectively) was typical of a modified indoline chromophore. The UV spectrum remained unchanged in dilute acid or alkali, but in 50% perchloric acid the spectrum was characteristic of a 3H-indolium structure ($\lambda_{max}^{50\%HClO_4-EtOH}$ 238, 242, 297 nm; log ϵ: 3·71, 3·69; 3·82 respectively). The characteristic shift of the absorption maxima in a strongly acid medium, and the color reactions of the alkaloid suggested that picralstonine possessed a 2-alkoxy indoline chromophore similar to that in picrinine.[5–7] The IR spectrum of picralstonine showed the presence of a hydrogen-bonded –NH– (broad band at 3049 cm^{-1}), an *o*-disubstituted benzene (1621, 763 and 748 cm^{-1}) and a carbomethoxyl function (1742 and 1171 cm^{-1}). The presence of a carbomethoxyl was confirmed by functional group analysis which showed the presence of one methoxyl group, and by saponification experiments. Hydrolysis of picralstonine with ethanolic KOH gave an amorphous acidic material, which on remethylation with diazomethane furnished the original compound, and another substance which was identified as picrinine.

The MS of picralstonine was almost identical with that of the isomeric picrinine (which possesses the same functional groups). However, it differed from this alkaloid in physical properties ($[\alpha]_D$, m.p., TLC behavior). Hence it appeared that picralstonine had the same carbon–oxygen–nitrogen skeleton as picrinine, but was either stereoisomeric with the latter at C_{16} or had a different configuration at the C_{19}–C_{20} double bond. The formation of picrinine during saponification experiments supported the former possibility, which was confirmed by an epimerization of picrinine at C_{16}. When picrinine was refluxed with sodium methoxide in anhydrous MeOH for 4 hr and the reaction mixture worked up in the usual way, some picralstonine was obtained in addition to unchanged picrinine. On basis of the above observations picralstonine was assigned the structure (III) (16-epi-picrinine).

This structural assignment was further confirmed by the appearance of the –NH– band at 3049 cm^{-1} in the IR spectrum, instead of at 3448 cm^{-1} as in picrinine,[5,7] suggesting very strong hydrogen bonding involving this group in the former. This observation is explicable as an examination of the Dreiding models of picrinine and picralstonine shows that in the latter the C_{16}-carbomethoxyl is situated very close to the indoline –NH–, which allows the formation of a strong hydrogen bond.

EXPERIMENTAL

The m.ps were determined on the Kofler block and are uncorrected. The UV spectra were measured in 95% EtOH (aldehyde-free), the IR spectra in Nujol mull.

Isolation of the alkaloids. Air-dried and finely ground leaves of *A. macrophylla* (1·5 kg) were processed in two batches. In each batch, 750 g of the leaves were first defatted with light petroleum (b.p. 60–80°) in a Soxhlet apparatus for 30 hr. The defatted marc was then soaked in EtOH for 30 days. The ethanolic extract (3 l.) was concentrated under reduced pressure, and the concentrate (250 ml) was mixed with 5% aqueous citric acid (1 l.) for 12 hr. The mixture was then filtered through a bed of Celite. The filtrate was adjusted to pH 10 with ammonia solution and extracted with $CHCl_3$ (5 × 500 ml). The $CHCl_3$ extract was dried over anhydrous Na_2SO_4 and concentrated. The concentrate containing the alkaloid mixture was chromatographed over Brockmann alumina. The light petroleum–benzene (1:1 and 1:3) eluates were mixed together, and the major component in this fraction was isolated by preparative TLC using silica gel as adsorbent and an EtOAc–EtOH mixture (5:1) as the developing solvent. This compound was recrystallized from benzene as

fine needles (yield 0·003%), m.p. 185°, $[\alpha]_D^{25}$ +27° ($CHCl_3$). MS, m.m.p., co-TLC and superimposable IR spectra of the alkaloid with an authentic sample of affinisine, m.p. 191°, $[\alpha]_D^{25}$ +30° ($CHCl_3$), established its identity. The combined benzene–$CHCl_3$ (4:1, 2:3 and 1:1) eluates on TLC showed the presence of a number of bases. Rechromatography of these fractions on silica gel followed by preparative TLC using silica gel as adsorbent and an EtOAc–EtOH mixture (3:1) as developing solvent furnished two compounds. The first base crystallized as stout needles (yield 0·005%), m.p. 223–224°, $[\alpha]_D^{25}$ −44° ($CHCl_3$), from acetone. It was identified by comparison (MS, m.m.p., co-TLC and superimposable IR spectra) with an authentic sample of picrinine, m.p. 225°, $[\alpha]_D^{25}$ −47°. The other alkaloid, designated picralstonine, crystallized from acetone as colorless granules (yield 0·001%), m.p. 200°, $[\alpha]_D^{25}$ −90° ($CHCl_3$). UV: λ_{max}^{EtOH} 234, 286 nm (log ϵ: 3·81, 3·40) $\lambda_{max}^{2\%HClO_4\text{-}EtOH}$ 232, 286 nm (log ϵ: 3·82, 3·39); $\lambda_{max}^{0.1\ N\ NaOH\text{-}EtOH}$ 234, 285 nm (log ϵ: 3·78, 3·36); $\lambda_{max}^{50\%HClO_4\text{-}EtOH}$ 238, 242, 297 nm (log ϵ: 3·71, 3·69, 3·82). IR; ν_{max}^{Nujol} 3049 (broad, –NH), 1742 and 1171 (–CO_2CH_3), 1621, 763, 748 (*o*-disubstituted benzene) cm^{-1}. MS: M^+ 338 (38%); *m/e* 321 (17%, M–OH), 320 (69%, M–H_2O), 279 (20%, M–CO_2CH_3), 261 (44%, M–CO_2CH_3–H_2O), 247 (15%), 239 (100%), 182 (23%), 181 (28%), 180 (32%), 170 (19%), 169 (20%), 168 (38%) and 167 (27%). (M^+—338·162332, OMe—8·98%; $C_{20}H_{22}O_3N_2$ required MW 338·163032, OMe (one)—9·18%).

Saponification of picralstonine. Picralstonine (5 mg) was refluxed with 10% ethanolic KOH (5 ml) for 12 hr, and on addition of 5 ml H_2O, the pH was adjusted to 6 with dilute HOAc. The residue obtained on evaporating this solution was leached with hot anhydrous MeOH. On evaporation this solution gave a white amorphous solid, which did not move on a TLC plate [adsorbent silica gel, developing solvent EtOAc–EtOH (3:1)]. The amorphous acidic material thus obtained, was dissolved in 5 ml of anhydrous MeOH, and an excess of an ethereal solution of diazomethane was added to it. TLC analysis of the reaction product showed the presence of two components, which were separated by preparative TLC and identified as picrinine and picralstonine respectively, by comparison (co-TLC and MS) with authentic samples.

Epimerization of picrinine. Sodium (200 mg) was added to anhydrous MeOH (10 ml), and the mixture was warmed for 30 min. Picrinine (100 mg) in anhydrous MeOH (15 ml) was then added, and the resulting solution was refluxed for 4 hr. Concentration of this solution to 3–4 ml, addition of H_2O (20 ml) and extraction with $CHCl_3$ (4 × 15 ml) furnished a mixture of two bases which were separated by preparative TLC. The major component (80 mg) was identified as picrinine, and the minor component (8–9 mg) as picralstonine by comparison (m.m.p., co-TLC and superimposable IR spectra) with authentic samples.

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