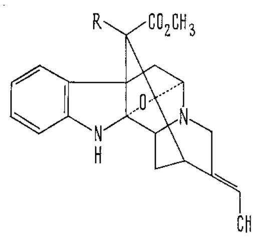
## Picralinal - A Key Alkaloid of Picralima Group from *Alstonia scholaris* R. Br.[1]

The reported hypotensive and anticancer activities[2] of the crude extracts of stem-bark of *Alstonia scholaris* R. Br. (Sanskrit: saptaparna; family: Apocynaceae) and our interest in the biosynthesis of non-tryptophan $C_{9-10}$ unit in indole alkaloids[3] led to the detailed investigation of the constituents of this plant. The present communication describes the isolation and constitution of a missing key alkaloid of picralima group from the leaves of *Alstonia scholaris* R. Br.

The concentrated alcoholic extract of the fresh leaves of the plant was macerated with 2*N* tartaric acid and then defatted with light-petroleum. The pH of the aqueous solution was brought down successively to 5 and 9. The weak and strong bases were extracted separately with benzene and ethyl acetate respectively. Column chromatography of the weak bases on neutral alumina gave picrinine (Ia)[4] and a new alkaloid. The ethyl acetate extract similarly gave a compound, mp 236°; $C_{21}H_{24}N_2O_3$ ($M^+$, 352) which was characterized as akuammidine[5].



(I) a: R = H
b: R = CHO
c: R = $CH_2OH$

The new alkaloid, $C_{21}H_{22}N_2O_4$ ($M^+$, 366), mp 179–180°, $[\alpha]_D^{30}$ – 179.7° ($CHCl_3$; c, 1.78); showed an UV-spectrum at $\lambda_{max}$ (EtOH) 237 and 293 nm (log $\varepsilon$ 3.87 and 3.43 respectively) characteristic of an indoline system. The IR-spectrum ($CHCl_3$) supported the presence of an NH group (3370 $cm^{-1}$) and indicated an ester C=O (1730 $cm^{-1}$, shoulder) and an aldehyde function (1720 and 2750 $cm^{-1}$). The NMR-spectrum ($CDCl_3$) showed the following signals: $\tau$ 8.49, dd (J 2 and 7 Hz), 3, $=C\begin{smallmatrix}\diagup CH_3\\ \diagdown H\end{smallmatrix}$; 6.30, s, 3, $CO_2CH_3$; 5.15, d (J 2.5 Hz) 1, $\begin{smallmatrix}O\diagdown\\ N\diagup\end{smallmatrix}CH$; 4.59, bq (J 7 Hz), 1, $=C\begin{smallmatrix}\diagup CH_3\\ \diagdown H\end{smallmatrix}$; 4.47, bs, 1, N*H*; 2.56–3.44, m, 4 ar. *H*; 1.42, s, 1, *H*C=O.

Sodium borohydride reduction of the base afforded an alcohol, mp 194–195°. The NMR-spectrum of the latter was similar to that of the new base, except that the aldehydic proton singlet had been replaced by signals at $\tau$ 6.40 (2H) and 6.42 (1H) respectively corresponding to a $CH_2OH$ group. Further, treatment of this alcohol with aqueous alcoholic potassium hydroxide at 80° for 15 min readily gave a compound, $C_{20}H_{22}N_2O_3$, formed by the loss of elements of formaldehyde and identical with picrinine. It is obviously a retroaldol type of reaction involving $HO\cdot CH_2\cdot\overset{|}{\underset{|}{C}}\cdot CO_2CH_3$ grouping. The alcohol was thus identified as desacetylpicraline (Ic)[6].

The foregoing evidence led to the constitution (Ib) for the new base identical with picralinal prepared earlier by Britten and Smith[6] by the chromic acid oxidation of (Ic). This assignment received support from the conversion of picralinal to (Ia) on alkali treatment and by its mass spectrum which showed, apart from the $M^+$ peak, abundant ions at *m/e* 338, 337 (base peak, M-29), 307 (M-59), 277 [M-(29 + 59 + 1)], 239 (M-127, due to loss of OHC–C–$CO_2CH_3$ together with CO derived from C-5 and a H transfer), and 130.

In view of the present status of the biogenetic theory concerning indole alkaloids, picralinal would appear to be a key intermediate for the biosynthesis of several indole alkaloids (i.e., picraline, desacetylpicraline, vincaricine[7], picrinine and echitamine). The present isolation of this hitherto missing alkaloid provides an additional example of a natural product previously known as a semi-synthetic laboratory product[8,9].

*Zusammenfassung.* Aus den Blättern von *Alstonia scholaris* R. Br. sind Pikrinine (Ia), Akuammidine und ein neues, bisher in Pflanzen nicht gefundenes Alkaloid, Pikralinal (Ib) isoliert worden. Pikralinal ist eine Schlüsselsubstanz für die Biosynthese vieler Alkaloide des Pikralimatypus.

R. C. Rastogi, R. S. Kapil
and S. P. Popli

*Central Drug Research Institute, Lucknow (India), 8 April 1970.*

---

[1] Part IV in the series *Studies in Medicinal Plants*; Part III: F. Anwer, S. P. Popli, R. M. Srivastava and M. P. Khare, Experientia *24*, 999 (1968).

[2] M. L. Dhar, M. M. Dhar, B. N. Dhawan, B. N. Mehrotra and C. Ray, Indian J. exp. Biology *6*, 232 (1968).

[3] A. R. Battersby, J. C. Byrne, R. S. Kapil, J. A. Martin, T. G. Payne, D. Arigoni and P. Loew, Chem. Commun. (*1968*), 951; and references therein.

[4] A. Chatterjee, B. Mukherjee, A. B. Ray and B. Das, Tetrahedron Lett. (*1965*), 3633.

[5] R. H. F. Manske, *The Alkaloids* (Academic Press, London 1965), vol. 8, p. 131.

[6] A. Z. Britten and G. F. Smith, J. chem. Soc. (*1963*), 3850.

[7] Kh. T. Ilyasova, V. M. Malikov and S. Yu. Yunusov, Khim. Prir. Soedin *4*, 327 (1968).

[8] Communication No. 1507 from the Central Drug Research Institute, Lucknow.

[9] Since the submission of this manuscript, we have come across the work of D. A. Evans, J. A. Joule and G. F. Smith, Phytochemistry, *7*, 1429 (1968) wherein they have reported the isolation of picralinal from Rhyza stricta.

---

## Fluorescent Metabolites in Mold-Damaged Wheat Flour

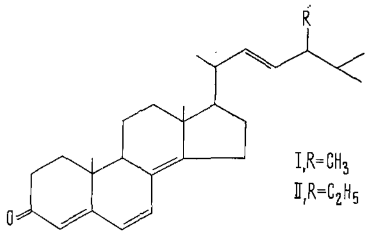
Cereals stored at elevated temperatures and high moisture levels show rapid increases in mold count and extensive lipid breakdown[1,2]. In the case of wheat flour, the breadmaking properties are seriously impaired and deterioration is accompanied by the formation of several fluorescent compounds of unknown structure[3]. The possibility that those compounds were fungal toxins[4] prompted further investigation.

Four wheat flours (with moisture contents of about 18%) stored for about 16 weeks at temperatures of 23, 30 or 37 °C showed a decrease in petroleum-ether-extractable lipids from 0.75–0.89% to 0.17–0.36% and an increase in mold count (chiefly *Aspergillus niger, A. candidus, A. versicolor,* and *A. flavus*) from about 100 to nearly 3 million per g of flour. Extraction with petroleum ether of 6 kg of mold-damaged flour yielded a lipidic fraction (12 g) from which the fluorescent material (60 mg) was isolated by chromatography on a series of 7 silicic acid and Sephadex LH-20 columns, and followed by crystallization from petroleum ether. Details of the isolation, purification, and characterization will be reported elsewhere.

Although chromatographically homogeneous, the fluorescent material (mp 105–107°) was found by mass spectrometry to consist of 2 major components with molecular weights 392 ($C_{28}H_{40}O$) and 406 ($C_{29}H_{42}O$) in the approximate proportions 70–75% and 20–25%, and it also contained (in earlier preparations) about 5% of $C_{28}H_{38}O_2$ (molecular weight 406).

In a final separation, the minor component was removed by rechromatography and recrystallization. The fluorescent material so obtained mp 107–109.5°, consisted of 2 components having formulae $C_{28}H_{40}O$ (ca. 75%) and $C_{29}H_{42}O$ (ca. 25%). The 2 samples of fluorescent material were indistinguishable in their spectroscopic properties except for the minor $C_{28}H_{38}O_2$ molecular ion in the mass spectrum of the first preparation and the presence of an additional absorption maximum, $\lambda_{max}$ 328 $E^{1\%}_{1\,cm}$ 900, in the UV-spectrum of that preparation.

The mass spectrum of the fluorescent material below mass 400 was identical with that of 4, 6, 8(14), 22-ergostatetraen-3-one (from D. H. R. BARTON) (I in Figure), hence the major component must be the ergostatetraenone. This was confirmed by the virtual identity of the NMR- and IR-spectra of compound (I) with those of the natural product. Furthermore, the reported[5,6] UV-spectrum of I ($\lambda_{max}$ 350 nm, $\varepsilon$ 27,100; $\lambda_{max}$ 282, $\varepsilon$ 7100; $\lambda_{max}$ 237, $\varepsilon$ 4700) matches closely the UV-spectrum of the purified preparation of fluorescent material ($\lambda_{max}$ 351 nm, $\varepsilon$ 25,800; $\lambda_{max}$ 282, $\varepsilon$ 5800; $\lambda_{max}$ 239, $\varepsilon$ 4100). Carbon, hydrogen, and oxygen elemental analysis on the purified preparation gave results in agreement with its formulation as a 3:1 mixture of $C_{28}H_{40}O$ and $C_{29}H_{42}O$ (Found: C, 85.58; H, 10.61; O, 4.07%. Calculated: C, 85.65; H, 10.32; O, 4.05). The ion *m/e* 406, $C_{29}H_{42}O$, must be due to a compound having one methylene group more than I. It is assigned structure II (Figure) on the following basis: a) the conjugated system must be the same as that in I, b) the extra $CH_2$ group must be in the side chain since the mass spectrum shows that all fragment ions which have lost the side chain have also lost the extra methylene group of the homolog, c) the olefinic protons in the side chain of I and of the natural product show complex but identical resonances at 4.8 $\tau$, hence methylation at $C_{22}$ or $C_{23}$ is excluded, and d) there is ample precedent in known steroids for the $C_{24}$-ethyl structure but there is no precedent for any of the other remaining possibilities.



Structural formulas of 4,6,8(14),22-ergostatetraen-3-one and its $C_{24}$-ethyl homolog.

It is of biogenetic interest that the methyl and ethyl homologs I and II are cogeners in the same biological system. Compound I has previously been isolated from a natural source[7] but $C_{29}$ steroids have hitherto only rarely been encountered as microbial metabolites. Methylation at $C_{24}$ to give ergosterol is known to involve methionine[8] as the carbon source. Recent evidence[9] suggests that the ethyl group in $C_{29}$ steroids is formed by stepwise methylation, methionine supplying both carbon atoms. The microbial synthesis of I and its homolog II is paralleled by the occurrence of homologous $C_{24}$-methyl and $C_{25}$-ethyl steroids in plants, for example campesterol and $\gamma$-sitosterol are both found in soybeans[10].

In a completely different field of endeavor our results suggest a simple test for storage deterioration, utilizing the characteristic fluorescence of I and II in detecting the commencement of mold damage. Preliminary tests have shown that the fluorescence can be easily detected by TLC on silica gel G in early stages of mold damage and that the fluorescence is apparently mold-species-dependent[11].

*Zusammenfassung.* Aus Schimmelpilzen (*Aspergillus niger, A. candidus* und *A. flavus*), die auf Weizenmehl wachsen, wurden die beiden fluoreszierenden Sterole 22-Ergostatetraen-3-on und dessen $C_{24}$-*Ä*thylhomologe isoliert.

R. D. DAFTARY, Y. POMERANZ,
R. G. COOKS and N. L. WOLFE

*Department of Grain Science and Industry,*
*Kansas State University, Manhattan (Kansas 66502, USA),*
*Barley and Malt Laboratory, Crops Research Division,*
*Agricultural Research Service, US Department of*
*Agriculture, Madison (Wisconsin 53705, USA), and*
*Chemistry Department, Kansas State University,*
*Manhattan (Kansas 66502, USA), 3 April 1970.*

---

[1] Y. POMERANZ, P. HALTON and F. G. PEERS, Cereal Chem. *33*, 157 (1956).

[2] Y. POMERANZ, R. D. DAFTARY, M. D. SHOGREN, R. C. HOSENEY and K. F. FINNEY, J. Agr. Food Chem. *16*, 92 (1968).

[3] R. D. DAFTARY and Y. POMERANZ, J. agric. Food Chem. *13*, 442 (1965).

[4] G. N. WOGAN, *Mycotoxins in Foodstuffs* (MIT Press, Cambridge, Mass. 1965).

[5] J. ELKS, J. chem. Soc. (*1954*), 468.

[6] D. H. R. BARTON and T. BRUUN, J. chem. Soc. (*1951*), 2728.

[7] H. MORIMOTO, I. IMADA, T. MURATA and N. MATSUMOTO, Annalyt. Chem. *708*, 230 (1967).

[8] I. D. FRANTZ and G. J. SCHROEPFER, A. Rev. Biochem. *36*, 718 (1967).

[9] L. LENFANT, E. ZISSMAN and B. LEDERER, Tetrahedron Lett. (*1967*), 1049.

[10] E. HEFTMANN and E. MOSETTIG, *Biochemistry of Steroids* (Reinhold Publishing Corp., New York 1960).

[11] We thank Prof. D. H. R. BARTON, Imperial College, London, for the sample of 4,6,8(14),22-ergostatetraen-3-one.