potent inhibitor and acetylation of the 2-NH_2 group of XI to afford XII does not alter activity significantly.

A series of derivatives of XI was selected to test the effect of substitution in the benzenoid nucleus on inhibitory potency. The 4-mercapto analogs were not prepared for this study in view of the extreme difficulty encountered in the synthesis of the unsubstituted compound XIII (9). The addition of a chlorine in the 5-position (XV) did not alter activity, while the inclusion of a 5-CH_3 (XVII) was highly deleterious. Conversely, the 6-Cl (XVI) and 6-CH_3 (XVIII) derivatives had similar potencies to XI. The presence of a 6-CN (XXI) or 7-CF_3 (XIX) caused little change in the I_{50}'s, while the 6-NH_2 derivative (XX) was some sixfold less inhibitory than the parent compound, XI. Compounds XXII–XXIV were synthesized as analogs of the pteridines, I*a* and *b*. Both the 6-CH_2OH (XXII) and 6-CHO (XXIII) derivatives were excellent inhibitors, with XXII being three times more potent than its pteridine counterpart, I*a*. In addition, XXIII was fourfold more inhibitory than XXII (which was apparently not the case with respect to I*a* and I*b*) and also served as a slow substrate, apparently being converted into the 6-COOH derivative (XXIV) based upon spectrophotometric observations. As was the case in the pteridine series (2), the introduction of a 6-COOH group (XXIV) yielded a substantially less inhibitory compound.

The 5,6,7,8-tetrahydroquinazolines (XXV and XXVI) were examined together with isocytosine (XXVII) to determine whether a second ring fused to the 2-amino-4-hydroxypyrimidine moiety was required for good activity and, if so, whether this ring needed to be unsaturated. None of these compounds showed inhibitory effects at 10 μM, so this structural feature appears to be required for good inhibition.

CONCLUSION

From the results discussed, it is apparent that suitably substituted quinazolines, but not related 5,6,7,8-tetrahydroquinazolines, can act as potent inhibitors of xanthine oxidase, rivaling or even surpassing their highly potent pteridine analogs. Furthermore, structure–activity considerations indicate that the quinazolines more closely resemble the pteridines with regard to inhibition of this enzyme than the analogous purines or pyrazolo[3,4-*d*]pyrimidines such as III*a*. For example, 2,4-dihydroxyquinazoline (VIII) is far less inhibitory than the reported (1) activity for III*b*, while XIII is substantially more inhibitory than its purine counterpart 2-amino-6-mercaptopurine (1).

REFERENCES

(1) B. R. Baker, W. F. Wood, and J. A. Kozma, *J. Med. Chem.*, **11**, 661(1968).

(2) H. G. Petering and J. A. Schmitt, *J. Amer. Chem. Soc.*, **72**, 2995(1950).

(3) D. G. Priest and J. R. Fisher, *Arch. Biochem. Biophys.*, **146**, 391(1971).

(4) J. J. McCormack and D. M. Valerino, in "Chemistry and Biology of Pteridines," International Academic Printing Co., Ltd., Tokyo, Japan, 1970, p. 381.

(5) S. S. Parmar, C. Dwivedi, and B. Ali, *J. Pharm. Sci.*, **61**, 179(1972).

(6) G. B. Elion, A. Kovensky, G. H. Hitchings, E. Metz, and R. W. Rundles, *Biochem. Pharmacol.*, **15**, 863(1966).

(7) G. B. Elion, *Ann. Rheum. Dis.*, **25**, 608(1966).

(8) T. Spector and D. G. Johns, *J. Biol. Chem.*, **245**, 5079(1970).

(9) W. T. Ashton, F. C. Walker, III, and J. B. Hynes, *J. Med. Chem.*, **16**, 694(1973).

(10) J. Davoll and A. M. Johnson, *J. Chem. Soc. C*, **1970**, 997.

(11) A. Rosowsky, J. L. Marini, M. E. Nadel, and E. J. Modest, *J. Med. Chem.*, **13**, 882(1970).

ACKNOWLEDGMENTS AND ADDRESSES

Received January 8, 1974, from the *Departments of Biochemistry and Pharmaceutical Chemistry, Medical University of South Carolina, Charleston, SC 29401*

Accepted for publication March 8, 1974.

Supported in part by the National Institutes of Health General Research Support Grant to the Medical University of South Carolina, 5 S01 RR 05420 (to D. G. Priest).

The authors acknowledge the technical assistance of T. L. Wheeler and C. Donato with the enzymatic assays.

* Present address: Merck Sharp and Dohme Research Laboratories, Rahway, NJ 07065

ˣ To whom inquiries should be directed.

Chemical Constituents of *Piper sylvaticum* (Roxb) and *Piper boehmerifolium* (Wall)

P. K. MAHANTAˣ, A. GHANIM*, and K. W. GOPINATH‡

Abstract □ A phytochemical investigation of the roots and stems of *Piper sylvaticum* (Roxb) resulted in the isolation and identification of piperine, piperlonguminine, *N*-isobutyldeca-*trans*-2-*trans*-4-dienamide, and β-sitosterol. In addition, two unidentified compounds were detected. The stems of *P. boehmerifolium* (Wall) included piperine, but further investigation was not attempted.

Keyphrases □ *Piper sylvaticum* (Roxb)—phytochemical investigation of roots and stems, isolation and identification of piperine, piperlonguminine, *N*-isobutyldeca-*trans*-2-*trans*-4-dienamide, and β-sitosterol □ *Piper boehmerifolium* (Wall)—phytochemical investigation of stems, isolation and identification of piperine

Piper sylvaticum and *P. boehmerifolium* are members of the Piperaceae and are distinguished by perennial roots and branches creeping on the ground or rooting like ivy on trees. These plants are abundantly found in the upper and lower parts of Assam in India (1). In the Ayurvedic system of medicine in India, these roots have been used for their laxative, anthelmintic, and carminative properties and in bronchitis, diseases of the spleen, and the treatment of tumors (2). Although no phytochemical studies on

these species have appeared in the literature, the genus *Piper* has received considerable attention in recent years (3–6).

EXPERIMENTAL[1]

Extraction of Plant Material[2] and Isolation of Piperine—The air-dried, crushed roots and stems of *P. sylvaticum* (3 kg) were extracted with benzene in a soxhlet apparatus for 36 hr. The extract was concentrated to 50 ml of an oily residue (A) which, after refrigeration, gave a light-yellow product, mp 125°. After recrystallization from benzene, the product yielded needles, mp 128–129° [lit. (7) mp 130°]. The IR and NMR spectra of this compound were identical with that of an authentic sample of piperine isolated from *P. nigrum* (7). The identity of the compound as piperine was further established by a mixed melting-point determination with an authentic sample of piperine; the melting point was undepressed. In a similar procedure, piperine was isolated and identified from the air-dried stems of *P. boehmerifolium*.

Isolation of β-Sitosterol—After the isolation of piperine, the crude alkaloid mixture was chromatographed over neutral alumina[3] packed in a column. On elution with petroleum ether–ethyl acetate (95:5), a neutral crystalline compound was obtained. On recrystallization from methanol, the material gave a white solid, mp 136–138° [lit. (8) mp 136–137°]. While the product did not show any absorption in the UV region of 220–400 nm, the IR spectrum indicated characteristic signals at 3450 (OH), 2950 (C—H stretching of C—CH_3), 2880, 1460, 1050, and 950 cm^{-1}. The compound gave a positive Libermann-Burchard test for steroids. An acetate derivative was prepared, mp 130–132° [lit. (8) mp 134°]. The identity of the compound as β-sitosterol was further established by an IR spectrum which was superimposable with that of an authentic sample.

Isolation of Piperlonguminine—The material remaining on the column was further eluted with petroleum ether–ethyl acetate (9:1). The eluent was concentrated to yield a residue which, when spotted on silica gel G plates, developed with a solvent system composed of petroleum ether–ethyl acetate (1:1), and sprayed with Dragendorff's reagent, showed a single spot. On recrystallization from benzene–petroleum ether (2:1), the crude alkaloid gave a colorless crystalline material, mp 167–168°; UV: λ_{max} 245, 256, 307, and 340 nm (log ε 3.90, 4.02, 4.33, and 4.52); IR: 3275, 1645 (monosubstituted unsaturated carboxy amide), 1650 (extended conjugation), 1375, 1325, and 995 (isopropyl, methylenedioxy, and *trans*-configuration) cm^{-1}; NMR: τ 4.10 (2H, methylenedioxy), 3.20–3.04 (3H, aromatic), 3.99, 3.68, 3.25, 2.50 (4H, olefinic), 8.95 (6H, methyl, d, J = 6 Hz), 7.90 (isopropyl), and 6.55 (2H, methylene adjacent to NH). The product had identical UV, IR, and NMR spectra and an identical melting point with those of an authentic sample of piperlonguminine, which had previously been synthesized by the condensation of piperic acid chloride with isobutylamine (4).

Anal.—Calc. for $C_{16}H_{19}NO_3$: C, 70.6; H, 6.98. Found: C, 70.83; H, 7.55.

Isolation of *N*-Isobutyldeca-*trans*-2-*trans*-4-dienamide—Six spots were found by TLC of the crude alkaloid extract (A) on silica gel G plates, developed with a solvent system composed of petroleum ether–ethyl acetate (1:1). The sample was chromatographed over a column packed with neutral alumina[3], and chlorophyll was removed by elution with petroleum ether. The remaining extract was separated into different fractions on preparative TLC [145 g silica gel G, with petroleum ether–ethyl acetate (1:1) as the developing solvent]. Fraction 2, on washing with petroleum ether, afforded a crude alkaloid. On recrystallization from petroleum ether, the crude alkaloid yielded colorless needles, mp 66–68°; UV: λ_{max} 258 nm (log 4.81, conjugated system); IR: ν_{max} 997 (*trans*-double bond), 947 (2,4-diene system with *trans*-configuration), 3300 (NH), 1625 (C=O), and 1650 (>C=C<) cm^{-1}. The IR spectrum was superimposable with that of the authentic sample of *N*-isobutyldeca-*trans*-2-*trans*-4-dienamide (5).

Anal.—Calc. for $C_{14}H_{25}NO$: C, 75.28; H, 11.28. Found: C, 74.89; H, 11.54.

A low melting, unstable alkaloid and a terpene, mp 195°, were also isolated. Their structural elucidation is in progress.

REFERENCES

(1) U. N. Kanjilal, P. C. Kanjilal, R. N. De, and P. C. Das, "Flora of Assam," vol. IV, Govt. of Assam, Shillong, India, 1940, pp. 31–38.

(2) K. R. Kirtikar and B. D. Basu, "The Indian Medicinal Plants," vol. III, 2nd ed., L. M. Basu, Allahabad, India, 1933, pp. 2125–2131.

(3) A. Chatterjee and C. P. Dutta, *Sci. Cult.*, **29**, 568(1963).

(4) A. Chatterjee and C. P. Dutta, *Tetrahedron*, **23**, 1769(1967).

(5) K. L. Dhar and C. K. Atal, *Indian J. Chem.*, **5**, 588(1967).

(6) J. Singh, K. L. Dhar, and C. K. Atal, *Tetrahedron Lett.*, **1971**, 2119.

(7) R. H. F. Manske and H. L. Holmes, "The Alkaloids," vol. I, Academic, New York, N.Y., 1950, pp. 168–170.

(8) "Dictionary of Organic Compounds," vol. V, 4th ed, Eyre & Spottiswoode, London, England, 1965, p. 2902.

ACKNOWLEDGMENTS AND ADDRESSES

Received August 8, 1973, from the *Regional Research Laboratory, Jorhat-6, Assam, India.*

Accepted for publication March 5, 1974.

The authors are grateful to Dr. M. S. Iyengar, Director, Regional Research Laboratory, Jorhat, for his encouragement and for allowing P. K. Mahanta to work in the Regional Research Laboratory, Jorhat, Assam, India; Prof. T. R. Govindachari, CIBA Research Centre, Bombay, for microanalytical data; Dr. C. K. Atal, Regional Research Laboratory, Jammu, for supplying the IR spectrum of *N*-isobutyldeca-*trans*-2-*trans*-4-dienamide; and Dr. P. Parimoo, Regional Research Laboratory, Jorhat, for assistance in preparing the manuscript.

* Present address: Central Arid Zone Research Institute, Jodhpur, Rajasthan, India.

‡ Present address: Ranbaxy Laboratories, OKHLA, New Delhi-20, India.

ˣ To whom inquiries should be directed.

[1] All melting points are uncorrected. The maximim wavelength of absorption in the UV spectrum was determined on a Carl Zeiss Jena spectrophotometer in 95% ethanol. The IR spectra were recorded on a Perkin-Elmer model 237B in mineral oil. The NMR spectra were recorded with a Varian T-60 instrument in $CDCl_3$ with tetramethylsilane as the internal reference.

[2] *P. sylvaticum* was collected in Nowgong, Assam, during April 1969, and *P. boehmerifolium* was collected in the Kamrup district during February 1970. The identity of the plant material was confirmed by Mr. L. C. Rabha, Division of Medicinal and Economic Plants, Regional Research Laboratory, Jorhat-6, Assam. The voucher specimens of *P. sylvaticum* and *P. boehmerifolium* have been preserved at the Herbarium, Division of Medicinal and Economic Plants, Regional Research Laboratory, Jorhat-6, Assam, India.

[3] Brockmann activity I.