Phytochemistry, 1971, Vol. 10, pp. 883 to 885. Pergamon Press. Printed in England.

SHORT COMMUNICATION

THE STRUCTURE OF TAMBULETIN

J. B. HARBORNE
Phytochemical Unit, Department of Botany, University of Reading
and
P. LEBRETON and H. COMBIER
Départment de Biologie Végétale, Université de Lyon, France
and
T. J. MABRY and Z. HAMMAM
Cell Research Institute and Department of Botany, University of Texas at Austin, U.S.A.

(*Received* 13 *July* 1970)

Abstract—The structure previously advanced for the flavonol tambuletin isolated in 1947 from the seeds of *Xanthoxylum acanthopodium* (Rutaceae) has been shown to be incorrect. U.v., NMR, mass spectral and degradative studies indicate that tambuletin is not herbacetin 8-methyl ether (I) but is a glucoside of gossypetin 7 (or 8), 4′-dimethyl ether (II). A second compound, tambulin, obtained from the same seeds, appears from mass spectral data to be a gossypetin trimethyl ether.

INTRODUCTION

YELLOW flavonols are compounds with an extra hydroxyl (or methoxyl) function at the 6- or 8-position and are relatively rare in Nature.[1] Distributional studies have indicated that they are both of taxonomic and phylogenetic interest.[2,3] One such compound tambuletin (m.p. 269–71°, acetate m.p. 140–2°) was isolated from seed of *Xanthoxylum acanthopodium* (Rutaceae) in 1947 by Balakrishna and Seshadri[4] and its structure as 3,5,7,4′-tetrahydroxy-8-methoxyflavone (herbacetin 8-methyl ether) (I) was established by these authors by classical procedures and further confirmed by comparison of the natural pigment and its tetraethyl ether with synthetic materials.[5,6]

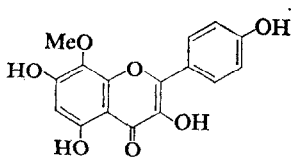
Another compound tambulin (m.p. 204°, acetate m.p. 164–5°) was earlier obtained from the same source by Bose and Bose,[7] who suggested that it was the 3,8,4′-trimethyl ether of herbacetin. Balakrishna and Seshadri[8,9] later found this structure to be incorrect, and suggested, on the basis of synthetic studies, that tambulin was herbacetin 7,8,4′-trimethyl ether. However, no direct comparison was made in this case between natural and synthetic materials and moreover attempts by the latter workers to obtain tambulin from *Xanthoxylum* seed yielded only tambuletin.

[1] J. B. HARBORNE, *Phytochem.* **4**, 647 (1965).
[2] J. B. HARBORNE, *Phytochem.* **8**, 177 (1969).
[3] J. B. HARBORNE and C. A. WILLIAMS, *Phytochem.* **9**, 2115 (1970).
[4] K. J. BALAKRISHNA and T. R. SESHADRI, *Proc. Indian Acad. Sci.* **25A**, 449 (1947).
[5] K. J. BALAKRISHNA and T. R. SESHADRI, *Proc. Indian Acad. Sci.* **26A**, 72 (1947).
[6] K. J. BALAKRISHNA and T. R. SESHADRI, *Proc. Indian Acad. Sci.* **26A**, 234 (1947).
[7] P. K. BOSE and J. BOSE, *J. Indian Chem. Soc.* **16**, 183 (1939).
[8] K. J. BALAKRISHNA and T. R. SESHADRI, *Proc. Indian Acad. Sci.* **26A**, 214 (1947).
[9] K. J. BALAKRISHNA and T. R. SESHADRI, *Proc. Indian Acad. Sci.* **26A**, 296 (1947).

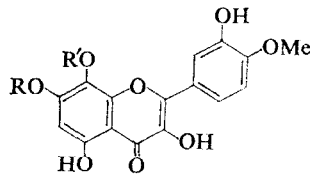
More recently, a flavonol aglycone was isolated from *Sedum acre* var. *sexangulare* (Crassulaceae) which from u.v., i.r., NMR and mass spectral studies could only be formulated as herbacetin 8-methyl ether (I).[10] However, although it had a similar m.p. (272°) to tambuletin, direct spectral and R_f comparison showed the two pigments to be different. Since the structure of the *Sedum* compound, which was given the name sexangularetin,[10] was firmly based on a full spectral analysis, a re-investigation of tambuletin seemed to be called for, in order to resolve the discrepancy.

RESULTS

The first suggestion that tambuletin was incorrectly formulated as a herbacetin derivative came from the u.v. spectral data[10] (the short wave band is characteristic for 3′,4′-*O*-substitution) and from demethylation under mild conditions which yielded gossypetin. The presence of a 3,5,7,8,3′,4′-hexahydroxyflavone nucleus in the compound was subsequently confirmed by NMR studies. Another property which did not fit the formulation of tambuletin as I was its unusually low R_f in butanol–acetic acid–water,[11] which was explained when the NMR spectrum revealed the presence in the molecule of a sugar unit. Subsequently, acid hydrolysis of tambuletin gave glucose and an aglucone, which is now formulated as gossypetin 7 (or 8), 4′-dimethyl ether (II).



(I)



(II) R = Me, R′ = H
(III) R = Me, R′ = glucose

Re-examination of tambuletin, using modern spectral methods, then showed that it could be formulated as the 8-glucoside of gossypetin 7,4′-dimethyl ether or, less likely, as the 7-glucoside of gossypetin 8,4′-dimethyl ether. The presence of the various structural features in III was arrived at as follows:

(1) A free 3-hydroxyl group was indicated by a comparison of the long wave band in the neutral spectrum of the aglucone (388 nm) with that of gossypetin (386 nm), i.e. there was no hypsochromic shift usually observed when the 3-hydroxyl of flavonols is substituted. Furthermore, the magnitude of the $AlCl_3$ shift in tambuletin (56 nm) was typical for flavonoids with a 3-hydroxy-4-carbonyl system.[12] (2) That the 5-hydroxyl was free was apparent from its general stability; 5-*O*-glycosylated and 5-*O*-methylated flavonols are unusually labile to hydrolytic cleavage.[13,14] (3) The presence of a 7-methoxyl was apparent when tambuletin failed to yield a recognizable A-ring fragment on reductive cleavage, which is characteristic of 7-methoxylated flavonoids.[15] (4) The presence of a 3′-hydroxyl 4′-methoxyl B-ring was

[10a] H. COMBIER, Thèse, Doct. Fac. Sci. Lyon (1968).
[10b] H. COMBIER, K. MARKHAM, H. AUDIER, P. LEBRETON, T. MABRY and M. JAY, *C.R. Acad. Sci. Paris* **266D**, 2495 (1968).
[11] J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, p. 57, Academic Press, London (1967).
[12] L. JURD, *Phytochem.* **8**, 445 (1969).
[13] J. B. HARBORNE, *Phytochem.* **6**, 1569 (1967).
[14] C. W. GLENNIE and J. B. HARBORNE, *Phytochem.* in press.
[15] H. M. HURST and J. B. HARBORNE, *Phytochem.* **6**, 1111 (1967).

indicated by the stability of the alkaline spectrum (i.e. since the 3-hydroxyl is free, see (1) above, the 4′-hydroxyl must be blocked) and by its failure to give a borate shift (i.e. 3′ or 4′-hydroxyl blocked). This was confirmed by the identification of 3-hydroxy-4-methoxyphenylpropionic acid as one of the products of reductive cleavage. (5) By elimination, the sugar residue in tambuletin is presumably located at the 8-position. Indeed, acid hydrolysis gave an aglucone, which was spectrally moderately unstable in alkaline solution, indicative of a 3,5,8-trihydroxyflavone system. The presence of a single glucose attachment was apparent from an R_f comparison of tambuletin with its aglucone and from NMR measurements. We, therefore, conclude that tambuletin does not have structure (I) but is the 8-glucoside of gossypetin 7,4′-dimethyl ether (III) or possibly the isomer with substituents at the 7- and 8- positions reversed. Structure (III) obviously still needs confirmation by synthetic means.

Measurement of the mass spectrum of tambuletin (see Experimental) showed the presence of a minor impurity (10–15 per cent of intensity of parent ion) with mass of 360, which corresponds in molecular weight to a gossypetin trimethyl ether; it is thus possible that Bose and Bose[7] were correct in thinking that a trimethyl ether was present in *Xanthoxylum* seed. However, it would appear from our data that any trimethyl ether present is based on gossypetin, and not herbacetin as suggested for tambulin. Therefore, until these seeds have been re-examined for their flavonoids, the natural occurrence of herbacetin 7,8,4′-trimethyl ether (tambulin) must be regarded as suspect. Unfortunately, *X. acanthopodium* only has a restricted distribution in northern India and our attempts to obtain seed of this plant for reexamination have so far failed.

EXPERIMENTAL

Material. An authentic sample of natural tambuletin was kindly provided by Professor T. R. Seshadri through the courtesy of Dr. T. Swain. It had m.p. 272–4° (recorded m.p. 269–271°).

Tambuletin had λ_{max} in EtOH at 259, 271* and 380 nm, unchanged on addition of NaOAc and NaOAc-H_3BO_3; with $AlCl_3$, max. were at 266, 348 and 436 nm and with NaOEt, at 422 nm (stable). In the mass spectrum, tambuletin showed the presence of a parent ion at 346 ($C_{17}H_{10}O_8$ requires mw 346) and minor peaks at 360 (+ CH_2, 14%), 328 (− CH_2, 10%) and 314 (−CHO, 10%). In the i.r. spectrum it exhibited carbonyl absorption at 1645 cm^{-1}. In the NMR spectrum (run in $CDCl_3$ as the trimethylsilyl ether), it gave signals at 7·88 (2′,6′-H), 6·99 (5′-H), 6·26 (6-H) and at 5·25 and 5·15 δ (sugar).

On demethylation for 4 hr at 120–140° with pyridinium chloride, tambuletin gave gossypetin, identified by spectral and chromatographic comparison with authentic material. On reductive cleavage, tambuletin gave 3-hydroxy-4-methoxyphenylpropionic acid as the only recognizable fragment. This was distinguished from the 3-methoxy 4-hydroxy isomer by difference in R_f on a two dimensional TLC plate run in 6% HOAc in $CHCl_3$ and in 45% EtOAc in benzene on SiO_2 and by difference in colour with Gibbs reagent (blue rather than pink).

Tambuletin aglucone was obtained on acid hydrolysis of tambuletin, together with glucose. It had λ_{max} in EtOH at 261, 278, 340 and 388 nm; with alkali, the spectrum showed max. at 291 and 336 nm but the long waveband decomposed with time. The aglucone had the following R_fs (× 100) (tambuletin values in parentheses): 60 (67) in Forestal, 51 (38) in *n*-BuOH–HOAc–H_2O (4:1:5) and 86 (86) in PhOH.

Acknowledgements—The authors thank Miss C. A. Williams for carrying out the reductive cleavage. J. B. H. thanks the Science Research Council and T. J. M. thanks the Robert A. Welch Foundation (Grant F-130) for financial support.