Phytochemistry, 1972, Vol. 11, pp. 2311 to 2312. Pergamon Press. Printed in England.

SHORT COMMUNICATION

THE STRUCTURE OF CALYCOPTERIN

E. RODRIGUEZ, G. VANDER VELDE and T. J. MABRY

The Cell Research Institute and Department of Botany, The University of Texas, Austin, TX 78712, U.S.A.

and

S. SANKARA SUBRAMANIAN and A. G. R. NAIR

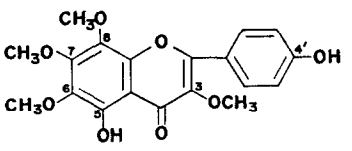
Department of Chemistry, Jawaharlal Institute of Postgraduate, Medical Education and Research, Pondicherry-6, India

(*Received* 24 *January* 1972)

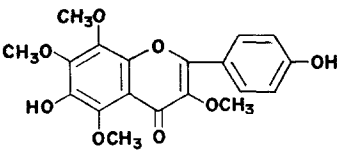
Key Word Index—*Calycopteris floribunda*; Combretaceae; calycopterin; 5,4'-dihydroxy-3,6,7,8-tetramethoxyflavone.

Abstract—NMR, UV, and MS spectral data confirm the structure of calycopterin as 5,4'-dihydroxy-3,6,7,8-tetramethoxyflavone.

CALYCOPTERIN, the major flavonol of *Digitalis thapsi* L. (Schrophulariaceae) and *Calycopteris floribunda* Lamk. (Combretaceae), was isolated both by Karrer[1] and Ratnagiriswaran *et al.*[2] in 1934. Structure I, which was subsequently proposed by Shah *et al.*,[3] was later confirmed by synthesis.[4] Nevertheless, Karrer[5] and Harborne[6] in their reviews of methoxylated flavonols assigned structure II to calycopterin (Harborne noted, however, that structure I



(I)



(II)

might be correct). Because of these ambiguities, we extracted a sample of new calycopterin from *C. floribunda* and recorded UV, MS and NMR data all of which are in accord with structure I.

[1] W. KARRER, *Helv. Chim. Acta* **17**, 1560 (1934).
[2] A. N. RATNAGIRISWARAN, K. R. SEHRA and K. VENKATARAMAN, *Biochem. J.* **28**, 1964 (1934).
[3] R. C. SHAH, V. V. VIRKAR and K. VENKATARAMAN, *J. Indian Chem. Soc.* **19**, 135 (1942).
[4] T. R. SESHADRI and K. VANKATESWARLU, *Proc. Indian Acad. Sci.* **24A**, 349 (1946).
[5] W. KARRER, *Konstitution und Vorkommen der Organischen Pflanzenstaffe*, Birhauser Verlag, Basel, Switzerland (1958).
[6] J. B. HARBORNE, *Comparative Biochemistry of Flavonoids*, Academic Press, New York (1967).

Spectral Data

The NMR spectrum of the trimethylsilyl ether of calycopterin indicated that the compound was a tetramethyl ether derivative: singlets for 4 methoxyl groups at 4·1–3·7,* aromatic protons at 8·2 ($J = 9$ Hz) and 7·0 ($J = 9$ Hz) for the H-2′–6′ and H-3′ -5′ protons, respectively. In benzene d_6[7,8] no significant upfield shifts were observed for the OMe resonances, ruling out substitution of the 4′ position.† That the 5-hydroxyl group was unsubstituted was evidenced by the presence of a signal for a hydrogen-bonded hydroxyl proton at 12·30.[9]

The presence of substituted oxygen functions at the 3, 6, 7 and 8 positions and hydroxyl groups at the 4′ and 5 positions was verified by UV spectral analysis: (1) In the presence of NaOMe, band I exhibited a bathochromic shift of 67 nm with an increase in intensity, typical for the presence of a 4′-hydroxyl group; (2) With $AlCl_3$–HCl, a band I bathochromic shift of 20 nm occurred which confirmed the presence of a 5-OH and a 6-O-substituent.[10]

The MS of calycopterin supported structure I: parent peak at *m/e* 374 ($C_{19}H_{18}O_8$ required 374), with a base peak at *m/e* 359 (M-15) diagnostic for 3,6,8-methoxylated flavones.[11,12] The fact that an intense ion at *m/e* 355 corresponding to the loss of H_3O (diagnostic for flavonols in which both the 3 and 5 positions are methoxylated)[12] is absent from the MS is also in accord with structure I for calycopterin.

EXPERIMENTAL

Two-dimensional chromatograms on Whatman 3MM paper were developed first in TBA (*t*-BuOH–HOAc–H_2O, 3:1:1) and then in 15% HOAc. The NMR spectra were recorded in CCl_4 and benzene-d_6 using tetramethylsilane as an internal standard. A standard set of six UV spectra were recorded. Fresh leaves (500 g) of *Calycopteris floribunda* (voucher specimen No. 13, deposited at the Jipmer Herbarium, India) collected from the Annamalia University Campus were extracted with ethanol. The ethanol concentrate was extracted with benzene yielding 300 mg of crude calycopterin. Recrystallization from EtOAc yielded 200 mg of pure calycopterin (identical with an authentic sample by m.m.p., TLC and acetate) m.p. 226° (uncorrected): R_f (TBA): 0·89; R_f (HOAc): 0·12; color test: purple (UV) to yellow (UV/NH_3). UV λ_{max} (MeOH): 233 sh, 278, 340 nm; λ_{max} (NaOMe): 260, 278, 407 nm; λ_{max} ($AlCl_3$): 245, 288, 310, 368 nm; λ_{max} ($AlCl_3$–HCl): 310, 360 nm: λ_{max} (NaOAc): 258 sh, 277, 402 nm; (NaOAc H_3BO_3): 280, 340 nm. The MS measurement of 5,4′-dihydroxy-3,6,7,8-tetramethoxy-flavone exhibited a parent peak at *m/e* 374 (80%) ($C_{19}H_{18}O_8$ requires 374), base peak at *m/e* 359 (M-CH_3) and other *m/e* peaks at 355 (3·7%), 345 (4·8%), 344 (6·2%), 343 (6·7%), 331 (2·5%), 329 (7·2%), and 301 (6·5%).

Acknowledgements—We thank Dr. K. Venkataraman, National Chemical Lab., Poona, India, for an authentic sample of calycopterin and Professor K. Rangaswami Ayyangar for the plant material. T. J. M. and coworkers thank the National Science Foundation (GB-29576X) and the Robert A. Welch Foundation (Grant F-130).

* Values are given in ppm (δ-scale) relative to TMS as an internal standard.

† Due to the presence of *ortho*-oxygenated constituents the 7-OMe exhibits only a small upfield shift (see Ref. 8).

[7] E. RODRIGUEZ, N. J. CARMAN and T. J. MABRY, *Phytochem* **11**, 409 (1972).
[8] R. G. WILSON, J. H. BOWIE and D. H. WILLIAMS, *Tetrahedron* **24**, 1407 (1968).
[9] T. J. MABRY, K. R. MARKHAM and M. B. THOMAS, *The Systematic Identification of Flavonoids*, Springer-Verlag, Heidelberg, New York (1970).
[10] J. A. MEARS and T. J. MABRY, *Phytochem* **11**, 411 (1972).
[11] J. H. BOWIE and D. W. CAMERON, *Austral. J. Chem.* **19**, 1627 (1966).
[12] D. G. I. KINGSTON, *Tetrahedron* **27**, 2691 (1971).