

BENZOFURAN AND BITHIOPHENES FROM ROOT CULTURES
OF *TAGETES PATULA*

FELIX J. PARODI, NIKOLAUS H. FISCHER,*

Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70803

and HECTOR E. FLORES

Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, Louisiana 70803

"Hairy root" cultures obtained upon transformation with *Agrobacterium rhizogenes* have been shown to express the pattern of secondary metabolites characteristic of the species from which they are derived (1). Because of the wide variety of biologically active compounds made in roots, this is an appropriate experimental system for studies on secondary metabolism and a potential source of new medicinal and agricultural chemicals (2,3). Fast growing root cultures from species of Asteraceae (Compositae) have been recently developed (4,5).

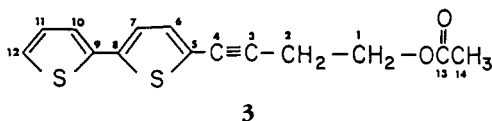
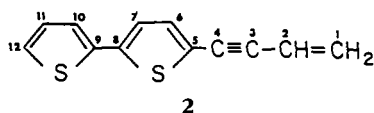
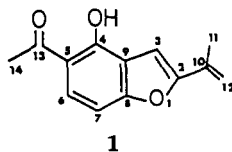
The chemical structures of the major components of the transformed root cultures of *Tagetes patula* L. (Asteraceae), 5-(4-acetoxy-1-butyryl)-2,2'-bithiophene [3], 5-(buten-1-nyl)-2,2'-bithiophene [2], and isoeuparin (5-acetyl-4-hydroxy-2-isopropenylbenzofuran [1] have been determined by spectroscopic methods (¹H nmr, ¹³C nmr, and ms). Inasmuch as high resolution nmr and ms data of 3 have not been previously reported, they

are summarized in the Experimental section.

EXPERIMENTAL

Several "hairy root" clones of *T. patula* were established at the Department of Plant Pathology at LSU in the spring of 1986 (5). A six-month-old clone of *T. patula*, at late exponential phase in a batch liquid culture, was used for extraction. A crude CH₂Cl₂ extract (30 mg) from 42 g of transformed "hairy roots" (5% dry matter) of *T. patula* was chromatographed by preparative tlc on Si gel with petroleum ether-CHCl₃ (2:1) providing seven highly uv-366 fluorescent bands. Analysis of these fractions afforded 3 (10 mg), 2 (4 mg), and 1 (3 mg) from bands 3, 2, and 1 (least polar), respectively.

5-(4-Acetoxy-1-butyryl)-2,2'-bithiophene [3], C₁₄S₂O₂H₁₂, gum; eims (70 eV) *m/z* (rel. int.) [M]⁺ 276 (9), [M - MeCO]⁺ 233 (0.2), [M - MeCOOH]⁺ 216 (100), [M - CH₂ - OAc]⁺ 203 (14), 184 (4), 171 (15), 158 (56), 127 (9), 115 (8), 95 (10), 69 (13), [Ac]⁺ 43 (52); ¹H nmr (400 MHz, CDCl₃) δ 4.22 (dd, 2H-1), 2.8 (dd, 2H-2), 7.04 (d, H-6), 7.00 (d, H-7), 7.16 (dd, H-10), 7.01 (dd, H-11), 7.22 (dd, H-12), *J*_{1,2} = 6.9, *J*_{6,7} = 3.8, *J*_{10,12} = 1.1, *J*_{10,11} = 3.7, *J*_{11,12} = 5.1 Hz; ¹³C nmr (100.13 MHz, CDCl₃) δ 62.09 (t, C-1), 20.75 (t, C-2), 75.15 (s, C-3), 90.57 (s,



C-4), 122.04 (s, C-5), 132.38 (d, C-6), 123.26 (d, C-7), 136.75 (s, C-8), 138.09 (s, C-9), 124.10 (d, C-10), 127.87 (d, C-11), 124.83 (d, C-12), 170.84 (s, C-13), 20.89 (q, C-14). The spectroscopic data of **1**, **2**, and **3** are in agreement with previously reported data (6-8).

The nmr spectral data were obtained by the use of multipulse and two-dimensional nmr techniques (9). Spectra and full details on the identification of these compounds are available on request from the authors.

ACKNOWLEDGMENTS

The authors are grateful to Mary W. Hoy and Julie J. Pickard for their expert technical assistance. This research was supported by the Louisiana Education Quality Support Fund (86-89)-RD-A-13 and the National Science Foundation Biotechnology Program (Project No. EET-8713078).

LITERATURE CITED

1. H.E. Flores and P. Filner, "Primary and Secondary Metabolism of Plant Cell Cultures," Springer-Verlag, Berlin, 1987, pp. 174-185.
2. J.D. Hamill, A.J. Parr, M.J.C. Rhodes, R.J. Robbins, and N.J. Walton, *BioTechnology*, **5**, 800 (1987).
3. H.E. Flores, "Use of Plant Cells and Organ Culture in the Production of Biological Chemicals," ACS Symposium Series 334, ACS, Washington, DC, 1987, p. 66.
4. H.E. Flores, M.W. Hoy, and J.J. Pickard, *TIBTECH*, **5**, 64 (1987).
5. H.E. Flores, J.J. Pickard, and M.W. Hoy, "Proceedings of International Conference on Naturally Occurring Acetylenes and Related Compounds," Elsevier, 1988, in press.
6. R. Sutfield, F. Balza, and G.H.N. Towers, *Phytochemistry*, **24**, 876 (1985).
7. J.M. Burke, R.T. Scannell, and R. Stevenson, *Phytochemistry*, **25**, 1248 (1986).
8. J.K.M. Saunders and J.D. Mersh, *Progr. Nucl. Magn. Reson. Spectrosc.*, **15**, 353 (1982).
9. G.A. Morris, *Magn. Reson. Chem.*, **24**, 371 (1986).

Received 2 November 1987