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SYSTEMATIC IMPLICATIONS OF CHROMENES AND BENZOFURANS FROM *ENCELIA* (ASTERACEAE)

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Abstract—Phytochemical analysis of *Encelia* species yielded numerous chromene and benzofuran derivatives. Systematic comparison of the chemical profiles revealed three distinct groups of species. The species affinities, as elucidated by the chromene and benzofuran patterns, largely paralleled those based on recent morphological and anatomical findings and are thought to reflect the phylogeny of the genus.

INTRODUCTION

Encelia Adans. comprises around 18 species of shrubby perennials inhabiting the arid and semi-arid parts of southwestern North America [1]. The distribution of the genus largely overlaps the Sonoran and Mojave deserts, where several species, notably *E. farinosa*, are widespread and dominant elements of the desert floras. Two species are disjunct in South America; one, *E. canescens*, grows along the coasts of Peru and Chile and in northern interior Argentina; the other, *E. hispida*, is endemic to the Galapagos Islands.

Encelia is a member of the tribe Heliantheae and has been assigned either to the subtribe Helianthinae [2] or Ecliptinae [3]. Robinson considers *Encelia* to belong to a group of related genera including *Enceliopsis*, *Geraea*, *Flourensia* and *Phoebanthus*; they share morphological features such as pale anther thecae and sterile rays [3]. Historically, this group of genera has been a difficult subject for systematic study. The generic boundaries have been repeatedly redefined; many species originally assigned to *Encelia* have been transferred to *Enceliopsis*, *Geraea* and even genera apparently outside this group, such as *Simsia* and *Helianthus* [1]. Reproductive barriers are lacking within the genera, while intergeneric hybrids have sharply reduced fertility ([4, 5]; C. Clark and D. W. Kyhos, unpublished results).

As part of a larger investigation of this group of genera, we have studied *Encelia* with emphasis on its chromene and benzofuran chemistry. Chromenes and benzofurans are prominent natural products of many genera of the Asteraceae and are especially common in the tribes Astereae, Eupatorieae, Heliantheae, Inuleae and Senecioneae [6, 7]. Whereas the presence of these compounds in species of *Encelia* has occasionally been reported before [8–10], this study provides a comprehensive analysis on the distribution and systematic implications of chromenes and benzofurans in this genus.

RESULTS AND DISCUSSION

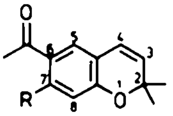
Chromenes and benzofurans are among the major natural products of *Encelia* and may in some species account for up to 5% of the dry weight of leaves and stems. The compounds usually occur in all organs analysed, including roots, stems, leaves, inflorescenses and achenes [11]. Within the plant tissues the compounds are stored in resin ducts, as was demonstrated by chemical analysis of the resin of *E. farinosa* and by fluorescent microscopic analysis of the plant tissues [12].

To obtain reproducible and reliable information on the chromene and benzofuran content and patterns within the genus, equal amounts of air-dried leaves and stems of each taxon were extracted separately. The extracts were reduced in volume and used for comparative TLC or HPLC. All major chromenes and benzofurans detected (compounds **1–14**) were consecutively isolated from bulk samples and identified spectroscopically. Comparison of the chemical data obtained revealed three distinct groups that could be clearly distinguished by their chromene and/or benzofuran constituents (Table 1).

The largest group, Group I, consists of 11 taxa that are characterized by an abundant accumulation of chromenes and benzofurans, up to a total concentration of 2–5% of the dry weight. The chromene and benzofuran patterns of all taxa of Group I proved to be remarkably homogeneous, with simple compounds like encecalin (**3**) or euparin (**4**) predominating. However, all taxa of this group also elaborated unusual dimers that regularly consisted of a euparin and encecalin moiety. Such dimers have only been isolated from *Encelia* so far [13] and clearly demonstrate the close relationship of the taxa in this group. The dimers reached their highest concentrations in the stems; in the leaves they were sometimes present only as trace amounts.

The observed chemical patterns proved to be consistent when several populations of one species were compared (Table 1), even though there were great differences in the amounts of the compounds accumulated. Ten samples of *E. farinosa* collected within 2 days in August 1984, along a west–east transect from Palm Springs, California to Tempe, Arizona, revealed a gradual decline in the concentration of encecalin, resulting in a ten-fold difference between the eastern and western extremes (Figs 1 and 2).

Group II consists of four taxa that show a markedly reduced capacity to accumulate chromenes and benzo-



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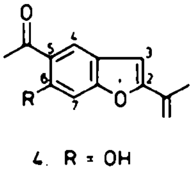

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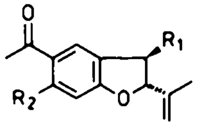
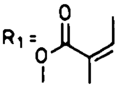
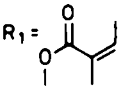
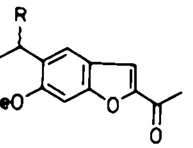
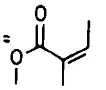
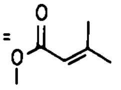


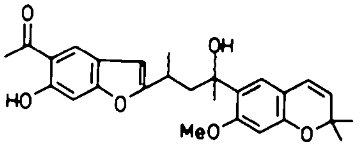
6 R_1 =  , R_2=H

7 R_1 =  , R_2=OH



8. R = 

9. R = 



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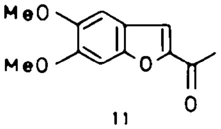
furans. Compounds like encecalin (3) or euparin (4) produced so abundantly by the taxa of Group I are, with the exception of *E. tenuifolia*, only trace compounds barely visible on TLC plates (Table 1). Dimers were missing totally. All the taxa of Group II, however, elaborated new esterified 2,5-diacetylbenzofuran derivatives (8, 9) [14]. These novel compounds provided evidence of the affinities of all taxa in this group.



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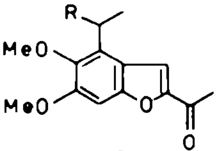
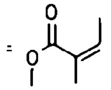
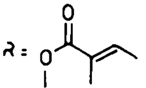
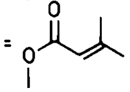


12 R = 

13 R = 

14. R = 

Group III consists of only two taxa. As in Group II, there was a marked trend towards reduction of the capacity to accumulate chromenes and benzofurans. Again only trace amounts of encecalin were detected (Table 1). But in contrast to Group II, no diacetylbenzofurans were present, allowing a clear distinction between these last two groups. The chemical data proved again to be stable when different populations of the taxa were compared (Table 1).

The remaining two taxa, *E. stenophylla* and *E. scaposa*, both elaborated compounds that were not observed in any of the previously described taxa. Esterified derivatives of tremetone and hydroxytremetone (6, 7) were isolated from *E. stenophylla* (Table 1). *E. scaposa* yielded several novel benzofuran derivatives (11–14). The spectroscopic elucidation of the structures will be published elsewhere (M. Breuer, P. Proksch and H. Budzikiewicz, in preparation). One compound (11) was a 2-acetylbenzofuran derivative recently isolated from *Eupatorium sternbergianum* [15]. Compounds 12–14 were novel esterified benzofuran derivatives. The acid substituents (angelic, senecic and tiglic acids) were esterified via the hydroxyethyl group at C-4. These compounds were further characterized by an acetyl substituent at C-2 of the furan ring. The unusual nature of the benzofurans (no acetyl group present at the aromatic ring of 11, hydroxyethyl group present at C-4 of compounds 12–14) clearly differentiated *E. scaposa* from the rest of the taxa analysed in this study.

These chemical data agree very well with morphological features to substantiate general ideas of phylogenetic relationship. The presence of the unique dimer in Group I suggests that it is a monophyletic group, and this is supported by the presence in all the members of ray corollas that reflect UV light (visible to insect pollinators) [16] and of a unique moniliform uniseriate trichome [17, 18].

The reduced capacity of Groups II and III to accumulate chromenes and benzofurans is a unique feature, at least among *Encelia* and its relatives, suggesting that these groups taken together are also monophyletic. This, too, is supported by morphology, all members having a

Table 1. Distribution of chromenes and benzofurans in *Encelia**

Species	Collections†	Compounds
GROUP I		
E. californica Nutt.	6, 115, 123, 166	1, 2, 3, 4, 5, 10
E. canescens Lam.	267, 268, 273, 281	1, 2, 3, 4, 5, 10
E. conspersa Benth.	189	1, 2, 3, 4, 5, 10
E. "densifolia" (undescribed sp.)	184	1, 2, 3, 4, 5, 10
E. farinosa A. Gray in Torr var. *farinosa*	1, 2, 3, 4, 5, 6, 7, 8, 9 10, 13, 18, 37, 247, 248	1, 2, 3, 4, 5, 10
E. farinosa var. *phenicodonta* (S. F. Blake) I. M. Johnston	82-163, 8, 183, 186, 190, 202	1, 2, 3, 4, 5, 10
E. halimifolia Cav.	143	1, 2, 3, 4, 5, 10
E. × *laciniata* Vasey & Rose	82-164	1, 2, 3, 4, 5, 10
E. palmeri Vasey & Rose	58, 118	1, 2, 3, 4, 5, 10
E. radians Brandegee	203	1, 2, 3, 4, 5, 10
E. ventorum Brandegee	131	1, 2, 3, 4, 5, 10
GROUP II		
E. actoni Elmer	11, 108, 156	3 (tr), 8, 9
E. frutescens A. Gray var. *resinosa* Jones *ex* Blake	195, 198	3 (tr), 8, 9
E. ravenii Wiggins	164	3 (tr), 8, 9
E. "tenuifolia" (undescribed sp.)	200	3 (tr), 8, 9
GROUP III		
E. asperifolia (S. F. Blake) Clark & Kyhos	1	—
	106, 129	3 (tr)
	132	3 (tr), 4 (tr)
E. frutescens A. Gray var. *frutescens*	154, 210	3 (tr)
	174, 176	—
OTHER SPECIES		
E. scaposa A. Gray	187	11-14
E. stenophylla Greene	133	1, 4, 5, 6, 7

*The reported compounds were analysed in leaves and stems.

†Collection data of three-digit numbers can be obtained from C. Clark, those of one-digit and two-digit numbers from P. Proksch. Voucher specimens referring to the collection numbers reported have been deposited at CSPU.

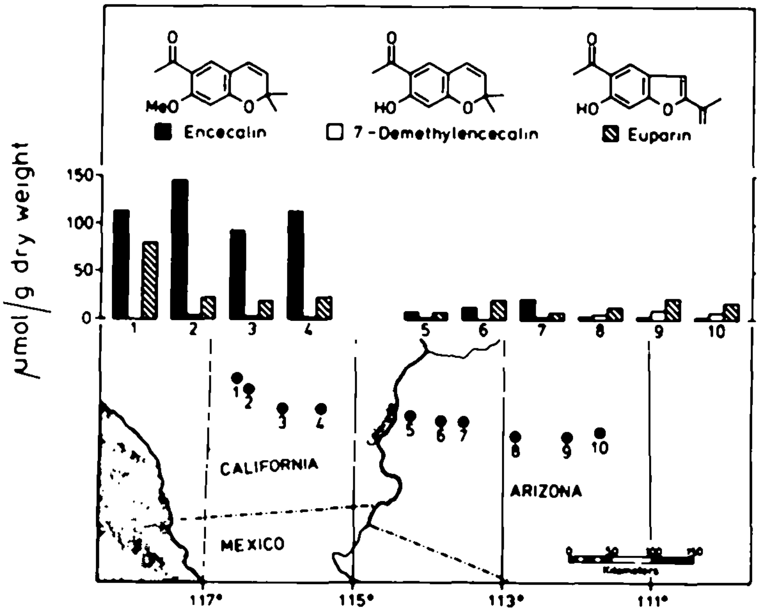


Fig. 1. Populational analysis of major chromenes and benzofuran from *Encelia farinosa*.

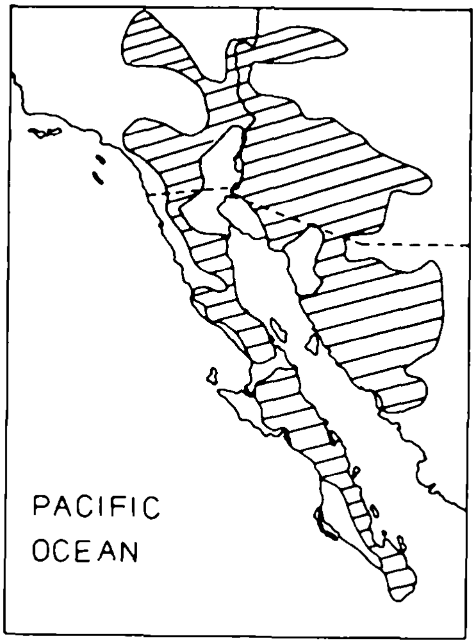


Fig. 2. Southwestern United States and adjacent Mexico. The hatched area resembles the natural distribution of *Encelia farinosa* (modified after ref. [23]). *E. farinosa* is gradually replaced by *E. farinosa* var. *phenicodonta* and *E. radians* (previously *E. farinosa* var. *radians*) from north to south in the peninsula of Baja California.

specialized development of the paleae in the fruiting head ([19]; C. Clark, unpublished results).

It would further appear that Group II itself is monophyletic, diagnosed by the unusual diacetylbenzofurans. However, at present morphology contradicts this assessment. Two of the taxa of Group II, *E. tenuifolia* and *E. frutescens* var. *resinosa*, share with both taxa of Group III a unique broad-based uniseriate trichome [17, 18]; the group possessing this character overlaps Group II, so at most one group can be monophyletic. It seems perhaps more reasonable to postulate that Group III secondarily lost the ability to synthesize diacetylbenzofurans, than to assume that a complicated trichome evolved twice independently.

The clear chemical differences between *E. frutescens* and its variety *resinosa* call into question the conspecificity of these taxa. Preliminary studies of their flavonoids also show significant differences (P. Proksch, in preparation). The taxa are morphologically quite distinctive as well, and specific status is being proposed for the variety *resinosa* (C. Clark, in preparation).

Encelia asperifolia is especially interesting in light of its apparent origin from the stabilization of diploid hybrids between *E. californica* (Group I) and *E. frutescens* (Group III) [20]. It shares with *E. frutescens* the inability to accumulate chromenes and benzofurans, and with *E. californica* the presence of UV-reflecting ray corollas.

The chemical differences between *E. stenophylla* and *E. scaposa* and the rest of the genus are paralleled by morphological differences (C. Clark, unpublished results), suggesting that these species, like many other species that have been removed from *Encelia* in the past, have their affinities elsewhere.

Whereas chromenes and benzofurans are already established as chemical markers which serve in delineating tribal affinities within the Asteraceae [6, 7, 21], this study for the first time demonstrates their importance for chemosystematic analyses at the subgeneric level. Furthermore, recent findings indicate this class of natural products to be a useful tool for gaining insight into the generic affinities between the related genera *Encelia*, *Enceliopsis*, *Flourensia* and *Geraea* (P. Proksch and C. Clark, in preparation).

EXPERIMENTAL

The compounds reported in this study were originally isolated from bulk samples of one collection of each taxon analysed and identified by their ^{1}H NMR and mass spectra. The spectral characteristics as well as details on the extraction and isolation procedures employed can be obtained from refs [11, 13, 14, 22]. Comparative analyses on the chromene and benzofuran patterns of the various populations studied of each taxon were conducted by TLC and HPLC of crude extracts [22].

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REFERENCES

1. Blake, S. F. (1913) *Proc. Am. Acad. Arts* **49**, 346.
2. Stuessy, T. F. (1977) in *The Biology and Chemistry of the Compositae* (Heywood, V. H., Harborne, J. B. and Turner, B. L., eds) p. 621. Academic Press, London.
3. Robinson, H. (1981) *Smithson. Contrib. Botany* **51**, 48.
4. Kyhos, D. W., Clark, C. and Thompson, W. C. (1981) *Syst. Botany* **6**, 399.
5. Kyhos, D. W. (1967) *Madroño* **19**, 33.
6. Proksch, P. and Rodriguez, E. (1983) *Phytochemistry* **22**, 2335.
7. Proksch, P. (1985) *Plant Syst. Evol.* **150**, 89.
8. Bjeldanes, L. F. and Geissman, T. A. (1969) *Phytochemistry* **8**, 1293.
9. Steelink, C. and Marshall, G. P. (1979) *J. Org. Chem.* **44**, 1429.
10. Bohlmann, F., Tsankova, E., Jakupovic, J., King, R. M. and Robinson, H. (1983) *Phytochemistry* **22**, 557.
11. Proksch, P. and Rodriguez, E. (1984) *Biochem. Syst. Ecol.* **11**, 179.
12. Proksch, P., Proksch, M., Weck, W. and Rodriguez, E. (1985) *Z. Naturforsch. Teil C* **40**, 301.
13. Proksch, P., Aregullin, M. and Rodriguez, E. (1983) *Planta Med.* **47**, 224.
14. Proksch, P., Breuer, M. and Budzikiewicz, H. (1987) *Phytochemistry* (in press).
15. Gonzalez, A. G., Fraga, B. M., Hernandez, M. G. and Garcia, V. P. (1982) *Phytochemistry* **21**, 1826.
16. Clark, C. and Sanders, D. L. (1987) *Madroño* (in press).
17. Clark, C., Thompson, W. C. and Kyhos, D. W. (1980) *Bot. Soc. Am., Misc. Ser. Publ.* 158.
18. Charest-Clark, N. (1984) *Crossoma* **10**, 1.
19. Charest-Clark, N. and Clark, C. (1984) *Am. J. Botany* **71**, 152.
20. Clark, C. (1983) *Am. J. Botany* **70**, 109.
21. Frohne, D. and Jensen, U. (1985) *Systematik des Pflanzenreichs*, p. 194. Gustav Fischer Stuttgart.
22. Proksch, P. and Rodriguez, E. (1982) *J. Chromatogr.* **240**, 543.
23. Bender, G. L. (1982) *Reference Handbook on the Deserts of North America*. Greenwood Press, London.