INSECT ANTIFEEDANT SESQUITERPENE LACTONES FROM SCHKUHRIA PINNATA: THE DIRECT OBTENTION OF PURE COMPOUNDS USING REVERSE-PHASE PREPARATIVE LIQUID CHROMATOGRAPHY

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Abstract - The structure of two germacranolides isolated from the East and West African plant Schkuhria pinnata have been determined by spectroscopic and chemical methods to be 1 (schkuhrin-I) and 2 (schkuhrin-II). These compounds were isolated in pure form directly from the crude aqueous methanol extract of whole plant in gram-scale by reverse-phase prep-LC. They exhibit antifeedant activity against the African army worm (Spodoptera exempta) and the Mexican bean beetle, (Epilancha varivestes), antimicrobial activity against some Gram-positive bacteria and in vivo cytotoxicity (KB test) at the level of 5.5 μg/ml.

Dedicated to Professor Tetsuo Nozoe on the occasion of his seventy-seventh birthday.

In our continuing search for insect antifeedant compounds[1,2], we have examined the East and West African medicinal plant Schkuhria pinnata (Compositae) from which two new bitter heliangolides schkuhrin-I[3], m.p. 59-61°C, and schkuhrin-II, m.p. 65-66°C, have been isolated.

Schkuhrins-I and -II exhibited antifeedant activity against the larvae of the African army worm Spodoptera exempta and the Mexican bean beetle Epilancha varivestis in the leaf-disk bioassay. In addition, they showed in vitro cytotoxicity at 5.5 μg/ml (KB test) and antimicrobial activity against certain Gram-positive bacteria.

To initiate chemical studies, the crude aqueous methanol extract of the whole fresh plant (ca. 16 g) was partitioned between various solvents resulting in the biological activity residing in the ether soluble fraction. The closely related components of this crude polar material were inseparable by silica gel chromatography. However, a separation could be achieved employing analytical reverse-phase hplc (μ-Bondapak C_{18}, 4 mm x 30 cm, methanol/water 55:45). Accordingly, a 6.6 g sample of the crude ether extract was injected onto a Jobin-Yvon Chromatospac Prep 500 packed with 1 kg of C_{18} stationary phase[4] (Figure 1). In less than one hour, the major fractions were eluted, collected, and evaporated to give gram quantities of pure compounds. As far as we know, this is the first example of the direct obtention of pure compounds from the crude ether

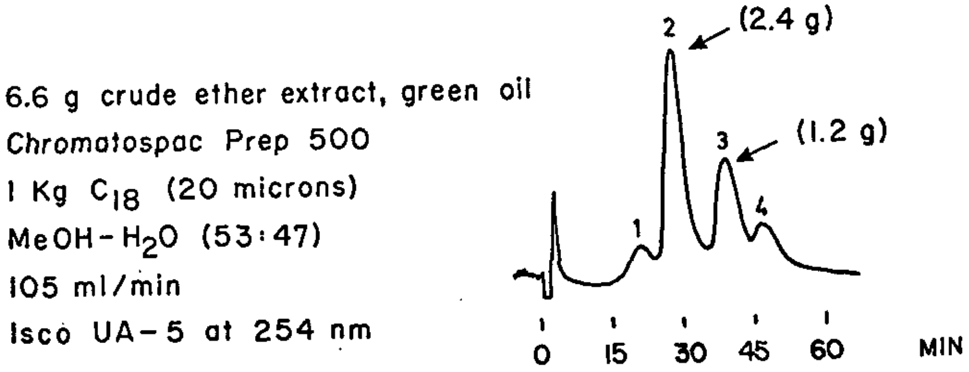
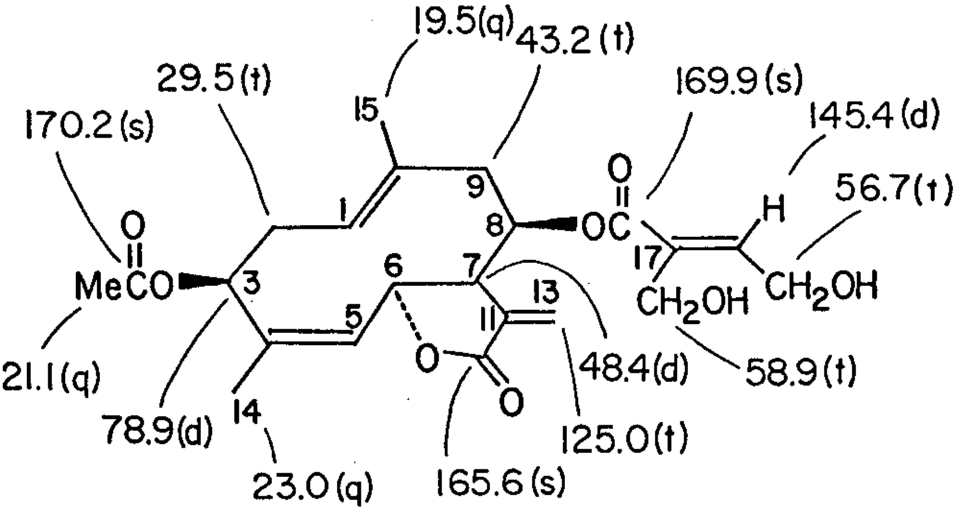


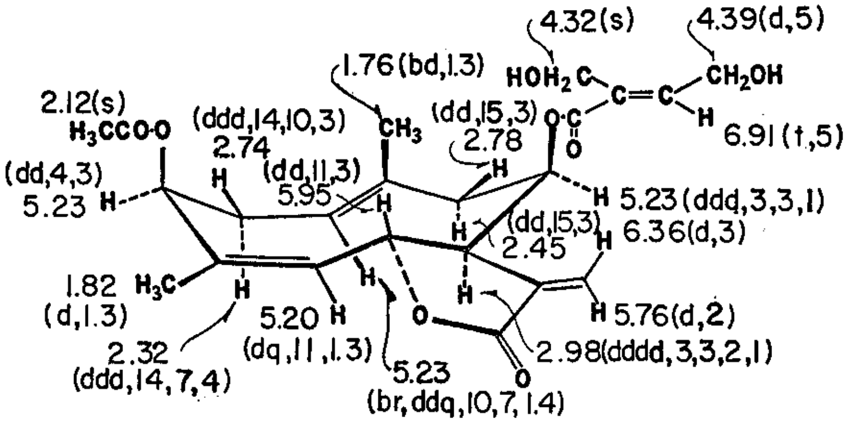
Fig. 1 Prep-lc of crude Schkuhria pinnata extract.

(polar) extract by reverse-phase prep-LC[5]. Spectroscopic and chemical studies on the bioactive compounds (fractions 2 and 3) have shown them to be trans-1, (10)-cis-4,5 germacradienolides.

Schkuhrin-I (**1a** and **1b**), $C_{22}H_{28}O_8$, possessed the following physical properties: UV(MeOH), end absorption; CD(MeOH) 259 nm ($\Delta\varepsilon$ + 0.74, $n\pi^*$), 215 ($\Delta\varepsilon$ -27.2); MS (CI-methane) 421 (MH^+); IR ($CHCl_3$) 3400 br (OH), 1755 (α,β-unsaturated γ-lactone), 1740 (ester), 1720 (α,β-unsaturated ester), 1660, 1250, 890 and 820 cm^{-1}. The CMR data (**1a**) showed the presence of three methyl, four methylene, and four methine carbon atoms together with eight olefinic and three carbonyl carbons[6].

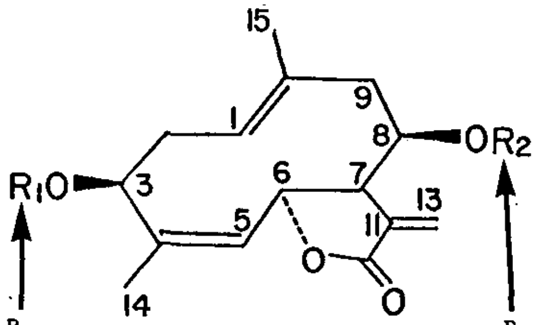


Structure **1a**: CMR in CDCl$_3$.
other peaks are:
C-8, -6 : 76.9(d), 76.0(d)
C-5, -1 : 126.3(d), 125.0(d)
C-4, -10, -11, -17 : 137.5(s), 136.7(s), 135.5(s), 131.4(s)



Structure **1b**: Schkuhrin-I, PMR in CDCl$_3$.

The presence of an α-methylene-γ-lactone moiety was indicated by the IR bands and the presence in the PMR of a characteristic pair of low field doublets at 5.76 (J 2Hz, H_b-13) and 6.34 ppm (J 3Hz, H_a-13), and appropriate signals in the CMR, particularly the triplet at 125.0 ppm (C-13). Due to the signal overlap, further conclusions could not be drawn from the PMR data. However, sequential addition of Eu(dpm)$_3$ to the CDCl$_3$ solution drastically spread out the congested spectrum, and extensive double resonance experiments on this solution led to the proton assignments listed in stereostructure 1b. The magnitudes of the induced chemical shifts indicated complexation of the lanthanide reagent with the ester hydroxyl functions as expected. The positions of the ester groups at C-3 and C-8, and the 6,7 closure of the lactone ring



	R_1	R_2
2	$\underset{O}{\underset{\|}{OC}}-\underset{OH}{CH}-\underset{CH_3}{CH}-CH_3$	$\underset{O}{\underset{\|}{OC}}-\underset{CH_2OH}{C}=CH(CH_2OH)$
3	$OCOCH_3$	H
4	H	H
5*	$OCOCH_3$	H
6*	H	H
7*	3,8-diketo	

*:Michael-type MeOH addition to C-11/C-13

Structures 2 - 7.

were established by the observed shift of proton 8 in the PMR of 3 (obtained by K_2CO_3 hydrolysis of 1 in aqueous dioxane) which agreed with the induced changes of protons 3,6, and 8 with the europium shift reagent.

The configurations of the 1,10- and 4,5-double bonds were established by a combination of CMR data and NOE experiments. The CMR resonance of methyl groups attached to trisubstituted double bonds are governed partially by the operation of the γ-effect[7,8] which leads to upfield shifts for olefinic methyls having cisoid γ-carbons (i.e., trans-olefinic methyls). The resonances of the vinyl methyl groups in 1a were 23.0, characteristic of a methyl on a cis double bond, and 19.5 ppm, intermediate between the signal for a methyl on a cis and on a trans double bond. NOE experiments designed to clarify these assignments were hampered by the complex nature of the vinyl region containing protons 1,3,5, and 8; thus, the various derivatives 3-7 were prepared. The cis nature of the 4,5-double bond was revealed by the observation of a 10 % NOE on 5-H upon irradiation of the C-4 methyl group in 5 (obtained by treatment of 1 with NaOMe/MeOH). In the 3,8-diketo derivative 7, obtained by Jones oxidation of 6, the CMR signal of the C-10 methyl was at 16.1 ppm; this high-field chemical shift established the 1,10-double bond configuration as trans. With the double bond configurations known, the stereochemistry at positions 3,6 and 8 could be assigned on the basis of coupling constants and chemical shift arguments.

In all higher plant germacranolides for which the absolute stereochemistry has been established, the H-7 is alpha[9]. The values of $J_{5,6}$ (11 Hz) and $J_{6,7}$ (3-Hz) require that 5-H/6-H and 6-H/7-H have trans relationships (see stereostructure 1b), i.e., that the lactone is trans-fused with the 6β-proton. Molecular models show that the small value of $J_{7,8}$ (ca.1 Hz) require the ester side-chain to be β-oriented. These assignments are in agreement with the magnitudes of downfield shifts for 6-H (medium), 7-H (small) and 8-H (large) in the $Eu(dpm)_3$ spectrum of 1. Since in going from monoester 3 to diol 4 the 6β-H resonance shifts downfield from 5.85 to 6.25 ppm, the C-3 oxygen function must also be β-oriented. The coupling constants of the 3α-H(t, J 3Hz) with the C-2 protons also support this conclusion.

The PMR spectrum of schkuhrin-II (2), $C_{25}H_{34}O_9$, UV (MeOH) end absorption; CD (MeOH) 259 nm (Δε + 0.70, $n\pi^*$), 215!(Δε 28.5); MS(CI-methane) 479(MH^+); IR(KBr) 3400 br(OH), 1760 (α,β-unsaturated γ-lactone), 1740(ester), 1715 (α,β-unsaturated ester), 1660, 1260, 895 and 820 cm^{-1}, correlated directly with that of schkuhrin-I expect for the absence of the acetate methyl singlet, and the appearances of a resonance at 4.05 ppm(1H, J 4.5 Hz), additional hydroxyl proton at 3.35 (br, 3H) an additional proton at 2.20 ppm, and a 6H triplet (J 7 Hz) at 0.98 ppm. Spin decoupling experiments clearly showed these changes to be due to a 2-hydroxy-3-methylbutanoate ester which replaces the C-3 acetate of schkuhrin-I. To our knowledge this is the first example of this ester group occurring in a sesquiterpene lactone. Schkuhrin's-I and -II were correlated by the hydrolysis of 2 with NaOMe/MeOH to the diol 6 identical in all respects to that derived from 1. These are the first reported examples of sesquiterpene lactone compounds from the genus Schkuhria.

The trans-1(10), cis-4(5) germacranolide skeleton was first encountered in peucephyllin isolated from Peucephyllium schottii Gray by Begley et al.[10] and subsequently from several other Compositae[11,12]. In view of the great interest in α-methylene-γ-lactones as cytotoxic agents, it should be noted that according to the modified method[13] of the Ames Test[14] employing strain TA-100 of Salmonella typhimurium, 776 revertants per plate were counted at a concentration of 100 μg plate in the presence of S-9 mix[15,16].

Acknowledgement - We are grateful to Instruments SA for loan of the Chromatospac Prep 500 and to Mr. A. Lopes for preparation of the C_{18} packing material.

References and Footnotes:

1. I. Kubo and K. Nakanishi in Host Plant Resistance to Pests ACS Symposium Series No. 62, P. A. Hedin (ed.) Washington, D. C.: American Chemical Society, 1977, pp. 165-178.
2. K. Nakanishi, in Natural Products and Protection of Plants, *Pontifical Academy of Sciences, Vatican City, 1976, p.185.*
3. Eucannibinolide [B.Drodz, H. Grabarczyk, Z. Samek, M. Holub, V. Herout, and F. Sorm, Collect. Czech. Chem. Comm. **37**, 1546 (1972)], whose double bond configurations were not rigorously determined, should be reformulated to **1** by comparison of their spectroscopic data. This reformulation was previously proposed on chemotaxomical grounds by a comparison with provincialin: W.

Herz and I. Wahlberg, *J. Org. Chem.* **38**, 2485 (1973).

4. D. Kingston and B. Gerhart, *J. Chrom.* **116**, 182 (1976).
5. For the first example of silica gel prep-LC applied to a crude hexane (non-polar) extract: K. Hostettmann, M. J. Pettei, I. Kubo, and K. Nakanishi, *Helv. Chim. Acta* **60**, 670 (1977).
6. These results were based on a combination of proton-noise decoupling, selective decoupling, and partially relaxed Fourier transform techniques: P. Zanno, I. Miura, K. Nakanishi, and D. Elder, *J. Amer. Chem. Soc.* **97**, 1975 (1975).
7. J. W. DeHann and L. J. M. vande Ven, *Org. Mag. Resonance* **5**, 147 (1973).
8. M. J. Pettei, F. G. Pilkiewicz, and K. Nakanishi, *Tet. Lett.* 2083 (1977).
9. W. Herz, *Isr. J. Chem.* **16**, 32 (1977).
10. M. J. Begley, G. Pattendèn, T. J. Mabry, M. Miyakado, and Y. Yoshioka, *Tet. Lett.* 1105 (1975).
11. For example: F. Bohlmann and L. Fiedler, *Chem. Ber.* **111**, 408 (1978).
12. Since submission of the original manuscript, Bohlmann et al. have isolated from the species *Isocarpha* an oily compound characterized as 20-hydroxychromolaenide, which turns out to be identical with schkurin-I: F. Bohlmann, P. K. Mahanta, A. A. Natu, R. M. King, and H. Robinson, *Phytochem.* **17**, 471 (1978). Although we have retained the name schkurin-I in this paper simply for the sake of convenience, it should be called 20-hydroxychromolaenide.
13. M. Nagao, T. Yahagi, Y. Seino, and T. Sugimura, *Cancer Lett.*

in press.

14. B. N. Ames, J. McCann, and E. Yamazaki, Mutation Res. **31**, 347 (1975).

15. M. Nagao, et al., to be published.

16. This work was supported by grants NSF INT 76-10311 and NIH AI-10187.

Received, 23rd December, 1978