Danksagung—Der Deutschen Forschungsgemeinschaft danken wir für die Förderung dieser Arbeit, Dr. E. Hutton, Jr. of Elkins für Pflanzenmaterial.

LITERATUR

1. Bohlmann, F., Dutta, L., Robinson, H. und King, R. M. (1979) *Phytochemistry* **18,** 1401.
2. Herz, W., de Groote, R., Murari, R. und Blount, J., (1978) *J. Org. Chem.* **43,** 3559.
3. Bohlmann, F., Mahanta, P. K., Suwita, A., Suwita, Ant., Natu, A. A., Zdero, C., Dorner, W., Ehlers, D. und Grenz, M. (1977) *Phytochemistry* **16,** 1973.
4. Kupchan, S. M., Kelsey, J. E., Muruyama, M., Cassady, J. C., Hemingway, J. C. und Knox, J. R. (1969) *J. Org. Chem.* **34,** 3876.

Phytochemistry, 1980, Vol. 19, pp. 1234–1236. © Pergamon Press Ltd. Printed in England. 0031-9422/80/0601-1234 $02.00/0

EUCANNABINOLIDE AND OTHER CONSTITUENTS OF *SCHKUHRIA VIRGATA**

WERNER HERZ and SERENGOLAM V. GOVINDAN

Department of Chemistry, The Florida State University, Tallahassee, FL 32306, U.S.A.

(*Received* 21 *September* 1979)

Key Word Index—*Schkuhria virgata*; Bahiinae, Heliantheae; Compositae; heliangolides; eucannabinolide; sesquiterpene lactones; pectolinarigenin; antileukemic activity.

Abstract—Isolation of eucannabinolide, its 3-isovaleroyl analog and pectolinarigenin from *Schkuhria virgata* (La Llave *et* Lex.) DC. is reported. The identity of eucannabinolide, which exhibits *in vivo* antileukemic activity, with 'hiyodorilactone A', '20-hydroxychromolaenide' and 'schkuhrin I' is discussed.

Schkuhria, an American genus of about 15 taxa [1], has recently been moved, together with its relatives in subtribe Bahiinae, from tribe Helenieae (Compositae) to Heliantheae [2]. We now report isolation of eucannabinolide (**1a**), its 3-isovaleroyl analog **1b** and pectolinarigenin from *Schkuhria virgata* (La Llave *et* Lex) DC.† and attempt to clear up some confusion concerning **1a** which exists in the literature. Eucannabinolide exhibited significant activity against lymphoxytic leukemia P 388 in the mouse.‡

The major lactone constituent of *S. virgata* was a gum, $C_{20}H_{28}O_8$ (high resolution MS), which on the basis of its mass, ^1H and ^{13}C NMR spectrum (Tables 1 and 2) was clearly the des-sarracinoyl derivative **1a** of provincialin (**1b**) [14]. In particular the chemical shift of H-3′, similar to that of tiglic acid, indicated the *E*-configuration for the five carbon-ester side chain and the chemical shifts of H-3′, H-4′, and H-5′ corresponded closely to those of appropriate signals in the ^1H NMR spectrum of eupaformosanin (**2**) whose structure has been established by X-ray crystallography [5].§

A substance to which structure **1a**, has been assigned has been isolated from several sources recently‖ and named variously 20-hydroxychromolaenide [7], hiyodori lactone A [8] and schkuhrin I [9]. Its spectral properties are indistinguishable from those of our material. Takahashi *et al.* [8] recognized a possible relationship of their 'hiyodorilactone A' to eucannabinolide, a gummy substance which was isolated previously [10] from *E. cannabinum* and was assigned [4, 11] structure **1a** on the basis of NMR data, but with side-chain stereochemistry unspecified in the printed formulas [9, 10]. Because the 4.00 ppm chemical shift reported [10] for H-4′ of eucannabinolide

* Research supported in part by a U.S. Public Health Service grant (CA-13121) through the National Cancer Institute.

† Our collection came from Honduras and was labelled *S. guatemalensis* Standl. *et* Steyermark. For synonymy of the only *Schkuhria* species occurring in central America see [3].

‡ Tests were carried out under the auspices of the Division of Cancer Treatment of the National Cancer Institute.

§ Liatripunctin [6] which contains the same five carbon-ester side chain was incorrectly drawn as the *Z*-isomer in ref. [5].

‖ *Isocarpha oppositifolia* (L) R. Br. [7], *Eupatorium sachalinense* Mak. [8] and an African collection of *Schkuhria pinnata* (Lam.) Kuntze [9].

Table 1. ^{1}H NMR spectra*

	1a	1c	1c†	3
H-1	5.27 *m*	5.27 *m*	4.67	5.20 *m*
H-2a	2.75 *m*	2.75 *m*	§	2.61 *m*
H-2b	2.31 *m*	2.3	§	§
H-3	5.27 *t*(3)‡	5.27 *m*	5.15 *dd*(4, 3)	5.26 *dd*(4.5, 3)
H-5	5.22 *dq*(10.5, 1.5)	5.20 *dq*(10.5, 1.5)	4.77 *d*	5.38 *dbr*(10.5)
H-6	5.96 *dd*(10.5, 2)	5.89 *dd*(10.5, 2)	5.89 *dd*	5.68 *dd*(10.5, 2)
H-7	3.01 *m*	2.99 *m*	2.22 *ddbr*	2.33 *ddbr*(5, 4)
H-8	5.27 *m*(4, 3, 1)‡	5.26 *m*(4, 3, 1)	5.11 *m*	4.07 *m*($W_{1/2}$ = 10)
H-9a	2.77 *dd*(15, 4)	2.73 *dd*(15, 3.5)	§	§
H-9b	2.47 *dd*(15, 3)	2.45 *dd*(15, 2.5)	§	§
H-13a	6.36 *d*(2.3)	6.35 *d*(2.5)	6.26 *d*	{3.67**}
H-13b	5.81 *d*(2)	5.78 *d*(2)	5.20 *d*	
H-14‖	1.82 *br*	1.80 *br*	1.74 *br*	1.82 *dbr*(1.5)
H-15‖	1.85 *d*(1.5)	1.84 *d*(1.5)	1.50 *br*	1.81 *d*(1.5)
H-3′	6.92 *t*(6)	6.90 *t*(6)	7.03 *t*	—
H-4′¶	4.40 *d*(6)	4.40 *d*(6)	4.03 *d*	—
H-5′¶	4.33	4.34	4.29	—
H-2″	2.14‖	2.26 *m*¶	§	2.19 *m*¶
H-3″		2.13 *m*	§	2.09 *m*
H-4″‖		0.98 *d*(6.5)	1.03 *d*	0.94 *d*(6.5)
H-5″‖		0.99 *d*(6.5)	1.06 *d*	0.96 *d*(6.5)
				2.76 *m*
				3.38(OMe)‖

* Run in $CDCl_3$ at 270 MHz unless otherwise specified with TMS as internal standard. Unmarked signals are singlets.

† In C_6D_6.

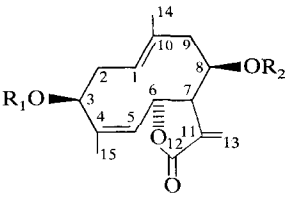
‡ *J*'s from spectrum in C_6D_6.

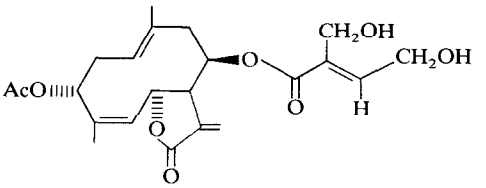
§ Obscured.

‖ Intensity three protons.

¶ Intensity two protons.

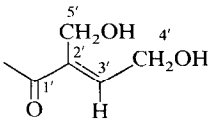
** Center of AB system, two protons.



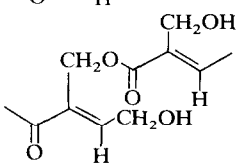


2

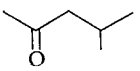
1a R_1 = Ac R_2 =



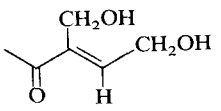
1b R_1 = Ac R_2 =

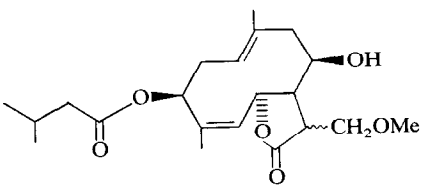


1c R_1 =



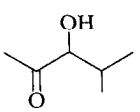
R_2 =





3

1d R_1 =



R_2 =

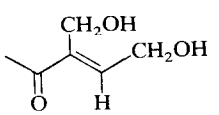


Table 2. ^{13}C NMR spectra*

Carbon	1a	1c
1	125.07 *d*†	125.24 *d*†
2	29.38 *t*	29.61 *t*
3	76.83 *d*‡	77.71 *d*‡
4	137.27	137.36
5	126.03 *d*†	126.21 *d*†
6	79.07 *d*‡	79.23 *d*‡
7	48.32 *d*	48.49 *d*‡
8	76.05 *d*‡	76.04 *d*
9	43.25 *t*	43.22 *t*
10	136.56§	136.57§
11	135.44§	135.37§
12	170.25	170.22
13	125.07 *t*	124.96 *t*
14	19.39 *q*	19.49 *q*
15	22.99 *q*	23.12 *q*
1′	165.49*q*	165.66
2′	131.09	131.44
3′	145.45 *d*	145.12 *d*
4′	58.78 *t*	58.88 *t*
5′	56.52 *t*	56.65 *t*
1″	169.90	171.86
2″	21.13 *q*	43.22 *t*
3″		25.35 *d*
4″		22.38 *q*
		22.35 *q*

* Run at 67.09 MHz in $CDCl_3$ with TMS as internal standard. Unmarked signals are singlets. All assignments tentative based on analogy and not verified by sford.

†‡§ Assignments may be interchanged.

differed from that observed for 'hiyodorilactone A' (4.31 ppm), the Japanese workers assumed that eucannabinolide was the *Z-isomer*, although in that case H-3′ should have occurred at a considerably higher field.

We therefore surmised the existence of an error in the literature or misprint with respect to the H-3′ frequency of eucannabinolide. While an authentic sample of the material from *E. cannabinum* was no larger available, correspondence with Dr. Holub revealed that the H-3′ signal of eucannabinolide actually occurs at 4.40 ppm and that in all other respects the properties of eucannabinolide and **1a** are essentially identical. Consequently, there is no doubt in our minds that the substances from *S. pinnata*, *S. virgata*, *E. sachalinense* and *I. oppositifolia* are identical with eucannabinolide and that the names 20-hydroxychromolaenide, hiyodorilactone A and schkuhrin I should be stricken from the literature.

A minor lactone constituent of *S. virgata* was the isovaleroyl analog **1c** (Tables 1 and 2). The distribution of the two ester functions over C-3 and C-8 was established by partial hydrolysis of **1c** to **3** in the manner first described for provincialin [4]. In the ^1H NMR spectrum of the product which retained the signals of the isovaleryl ester, the narrowly-split triplet of H-3 was unchanged, while the broad singlet of H-8 had moved upfield.

The constituents of *S. virgata* are thus very similar to those of an African collection of *S. pinnata* which yielded **1a** and **1d** [9]. *S. pinnata* is adventive in Africa and several varieties exist [1]. One of these is *S. pinnata* var. *virgata* ≡ *S. virgata* (La Llave *et* Lex.) DC., the central American variety whose only reliable difference from *S. pinnata* proper is said to be the nature of the pappus [1].

EXPERIMENTAL

Extraction of Schkuhria virgata. Aerial parts of *S. virgata* (La Llave *et* Lex.) DC. (*Schkuhria guatemalensis* Standl. *et* Steyermark) collected by Mr. Gustavo Cruz in the Fall of 1974 near Tegucigalpa, Honduras (voucher on deposit in herbarium of U.N.A.H., accession No. PR. 43868 of Medicinal Plant Resources Laboratory, U.S.D.A.) wt 6.0 kg, were extracted and worked up as usual [12]. The crude gum, wt 25.0 g, was preadsorbed on 35 g of silicic acid (Mallinckrodt 100 mesh), loaded on a column of silicic acid (300 g) packed in benzene and eluted with solvent of increasing polarity, 800 ml fractions being collected as follows: 1–4 (C_6H_6), 5–9 (C_6H_6–$CDCl_3$, 1:1), 10–12 ($CHCl_3$), 13–15 ($CHCl_3$–MeOH, 19:1). Fractions 5–8 on trituration with C_6H_6 yielded 0.05 g of pectolinarigenin, identified by mp, NMR, MS and direct comparison with an authentic sample. Fractions 10 and 11 (wt 1.37 g) showed one major spot on TLC; PLC (solvent MeOH–$CHCl_3$, 3:47) gave 0.36 g of gummy **1c**, $[\alpha]_D - 96.2°$ ($CHCl_3$, *c* 0.0322); CD curve (MeOH) $[\theta]_{252} + 1610$ (max); UV λ_{max}^{MeOH} 211 nm (ε 17 200); IR bands 3420 (*br*), 1750, 1720, 1710 cm^{-1} (Calc. for $C_{25}H_{34}O_8$: 462.2255. Found: 462.2255). Other significant peaks in the high resolution MS were at *m/e* (composition, %) 331 ($C_{20}H_{27}O_4$, 24.1), 246 ($C_{15}H_{18}O_3$, 23.8), 22 ($C_{15}H_{17}O_2$, 28.1), 228 ($C_{15}H_{16}O_2$, 44.2) and 85 (C_5H_9O, 100%).

Fractions 13–15 afforded 8.0 g of eucannabinolide (**1a**) whose properties corresponded to those given elsewhere [7–10]. ^1H and ^{13}C data are given in Tables 1 and 2.

Hydrolysis of **1c**. A solution of 50 mg of **1c** in 4 ml of MeOH and 1 ml of H_2O was stirred with 150 mg of K_2CO_3 for 30 min (N_2 atm), diluted with H_2O and extracted with $CHCl_3$.

Evaporation of the washed and dried extract furnished crude **3** which was purified by PLC (MeOH–$CHCl_3$, 1:9), yield 5 mg, IR bands 3430, 1755, 1735 cm^{-1} (Calc. for $C_{21}H_{32}O_6$: 380.2222. Found: 380.2198). Other significant peaks in the high resolution MS were at *m/e* (composition, %) 296 ($C_{16}H_{24}O_5$, 20.5), 278 ($C_{16}H_{22}O_4$, 24.3) and 246 ($C_{15}H_{18}O_3$, 21.2).

REFERENCES

1. Heiser, C. B., Jr. (1945) *Ann. Mo Bot. Gard.* **32,** 265.
2. Stuessy, T. F. (1977) in *Chemistry and Biology of Compositae* (Heywood, V., Turner, B. L. and Harbone, J. B., eds.). Academic Press, London.
3. Williams, L. O. (1976) in *Flora of Guatemala* (Nash, D. L. and Williams, L. O., eds.) Fieldiana: Botany, Vol. 24, Part XII, p. 279.
4. Herz, W. and Wahlberg, I. (1973) *J. Org. Chem.* **38,** 2485.
5. Lee, K.-H., Kimura, T., Haruna, M., McPhail, A. T. and Onan, K. D. (1977) *Phytochemistry* **16,** 1068.
6. Herz, W. and Sharma, R. P. (1975) *Phytochemistry* **14,** 1561.

7. Bohlmann, F., Mahanta, P. K., Natu, A. A., King, R. M. and Robinson, H. (1978) *Phytochemistry*, **17,** 471. These authors did not establish the distribution of the acetate and five carbon-ester side chain between C-3 and C-8.
8. Takahashi, T., Eto, H., Ichimura, T. and Murae, T. (1978) *Chem. Letters* 1345.
9. Pettei, M. J., Miura, I., Kubo, I. and Nakanishi, K. (1978) *Heterocycles* **11,** 471. These workers obtained **1a** in crystalline form by means of HPLC.
10. Drozdz, B., Grabarczyk, H., Samek, Z., Holub, M., Herout, V. and Šorm, F. (1972) *Collect. Czech. Chem. Commun.* **37,** 1546.
11. Holub, M. and Samek, Z. (1977) *Collect. Czech. Chem. Commun.* **42,** 1053.
12. Herz, W. and Högenauer, G. (1962) *J. Org. Chem.* **27,** 905.

Phytochemistry, 1980, Vol. 19, pp. 1237–1238. © Pergamon Press Ltd. Printed in England. 0031-9422/80/0601–1237 $02.00/0

KAURENOID DITERPENES FROM *STACHYS LANATA*

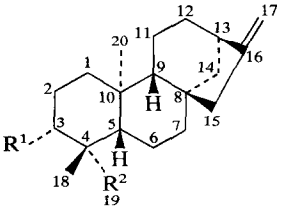
FRANCO PIOZZI,* GIUSEPPE SAVONA* and JAMES R. HANSON†

* Instituto di Chimica Organica, Universita di Palermo, Via Archirafi, Palermo, Italy;
† School of Molecular Sciences, University of Sussex, Brighton, Sussex, BN1 9QJ, U.K.

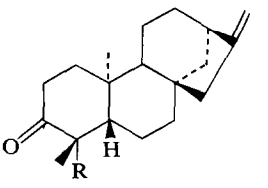
(*Received* 16 *August* 1979)

Key Word Index—*Stachys lanata*; Labiatae; *ent*-3β,19-dihydroxykaur-16-ene; *ent*-3β-hydroxykaur-16-en-19-oic acid.

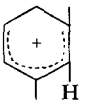
In continuation of our work on the diterpenoids of the Labiatae [1, 2], we have examined the medicinal herb, *Stachys lanata*. Extraction of the plant with acetone and chromatography afforded an acetoxy-acid, $C_{22}H_{32}O_4$ (**1**) together with a mixture of a diol, $C_{20}H_{32}O_2$ (**2**) and a hydroxy-acid, $C_{20}H_{30}O_3$ (**3**). The latter were separated by methylation and further chromatography. The ^{1}H NMR spectra of the esters **4** and **5** and the diol **2** contained resonances assigned to two tertiary methyl groups and an exocyclic methylene, suggesting that the compounds were kaurene derivatives. Oxidation of the hydroxy-ester **5** with 8 N CrO_3 gave a keto-ester **6**, whilst hydrolysis of the acetoxy-acid **1** and oxidation with 8 N CrO_3 gave a neutral nor-ketone, $C_{19}H_{30}O$ (**7**). The ^{1}H NMR spectrum of this ketone suggested that it contained a CH_3·CH group. Irradiation at δ 2.35 led to the collapse of the methyl doublet at 0.99 to a singlet. Thus, the hydroxy-acid was a β-hydroxy-acid. The multiplicity of the acetoxyl CH resonance (δ 4.52, $J = 4$ and 12 Hz) indicated that the oxygen substituent was equatorial. Comparison of the ^{13}C NMR spectra (Table 1) with the assignments for *ent*-kaur-16-ene (**8**) [3] led to the location of the hydroxyl group at C-3. The presence of oxygen functions on ring A was substantiated by a strong ion (**9**) at *m/e* 107.086 (C_8H_{11} requires 107.086) in the MS of **2**, **4** and **5**. Reduction of the methyl esters **4** and **5** afforded the diol **2** which proved to be identical to the known *ent*-3β,19-dihydroxykaur-16-ene [4, 5]. Hence, the acetoxy- and hydroxy-acids were **1** and **3** [6], respectively.



1 $R^1 = OAc$, $R^2 = CO_2H$
2 $R^1 = OH$, $R^2 = CH_2OH$
3 $R^1 = OH$ $R^2 = CO_2H$
4 $R^1 = OAc$, $R^2 = CO_2Me$
5 $R^1 = OH$, $R^2 = CO_2Me$
8 $R^1 = H$, $R^2 = Me$



6 $R = CO_2Me$
7 $R = H$



9

Whilst 19-oxidation of kaur-16-enes is common amongst the diterpenoids of the Compositae and Euphorbiaceae, 18-oxidation is more common amongst the tetracyclic diterpenoids of the Labiatae (e.g. *Sideritis* species). Stachysic acid, *ent*-6α-acetoxykaur-16-en-18-oic acid, has been obtained from *Stachys silvatica* [7].

EXPERIMENTAL

General experimental details have been described previously [1, 2].