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MIKANOKRYPTIN, A NEW GUIANOLIDE FROM *MIKANIA*

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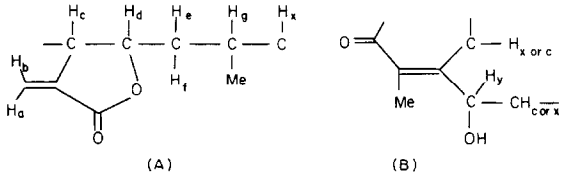
Key Word Index—*Mikania* sp.; Compositae; sesquiterpene lactones; guianolide; mikanokryptin; mikanolide.

Abstract—The isolation and structure determination of mikanokryptin, a stereoisomer of 11,13-dehydrogeigerin, from what appears to be a previously undescribed *Mikania* species is reported. *Mikania micrantha* HBK. yielded mikanolide and dihydromikanolide.

INTRODUCTION

In the course of our studies of *Mikania* species we have examined several collections from the Canal Zone. Two of these were authenticated as *Mikania micrantha* HBK and furnished mikanolide (**1**) [1, 2] and dihydromikanolide (**2**) as have other members of the Mikania *scandens* complex [3]. A third collection from the same vicinity, originally also assumed to be *M. micrantha*, yielded neither mikanolide nor mikanolide congeners, but a new sesquiterpene lactone of a different type. Because the collection has since been tentatively identified as representing a new species* we have named the new substance mikanokryptin and hereby report its structure (**3**a).



* Dr. E. L. Tyson who made the collection commented on the unsual flowering time and suggested that it might differ from authentic *M. micrantha*. Dr. Sidney McDaniel, Mississippi State University, to whom we are greatly indebted for other collections, identifications and valuable correspondence, initially inferred that the collection might represent *M. panamanensis* Robinson of which only a single rather immature specimen from the vicinity of Culebra, Canal Zone, was known at the time [7]. However, subsequent comparison with a fragment of the type and with authentic material of *M. panamanensis* collected by Dr. McDaniel indicated that the Tyson collection was clearly different not only from *M. micrantha*, but also from *M. panamanensis* and quite probably represents a previously undescribed species.

† Part of this unusually large shift is ascribable to a change of solvent from DMSO to $CDCl_3$.

DISCUSSION

Mikanokryptin, $C_{15}H_{18}O_4$, m.p. 248–250°, $[\alpha]_D$ +675°, has a UV spectrum characteristic of an enone (λ_{max} 240 nm, ϵ 17800) which is probably part of a cyclopentenone chromophore (IR bands at 1675 and 1632 cm^{-1}, cf. the UV and IR characteristics of geigerin (**4**a) and geigerin analogs) [8, 9]. The presence of a secondary hydroxyl group (IR band at 3420 cm^{-1}, NMR doublet in DMSO-d_6 which disappeared on addition of D_2O) was confirmed by conversion to an acetate **3**b; this was accompanied by a paramagnetic shift of a signal (hydrogen under hydroxyl) from 5·10 to 6·64 ppm.† The remaining two oxygen atoms of the empirical formula are contributed by the α,β-unsaturated lactone group in partial structure A (IR band at 1765 cm^{-1}) because the NMR spectrum of **3**a exhibits the typical low field doublets of H_a and H_b at 6·24 and 5·81 ppm. These disappeared on partial reduction of **3**b to **5**a in the presence of tris-(triphenylphosphine)-chlororhodium and were replaced by a new methyl doublet, but the enone chromophore remained unaffected. The location of the H_c resonance in a two-proton cluster at 3·23 ppm in the NMR spectrum of **5**b was established by double irradiation at the frequencies of H_a and H_b; further studies were complicated by the circumstance that even in the presence of shift reagents and at 270 MHz the two components of the cluster, H_c and H_x, remained superimposed.

Irradiation at the frequency of the two-proton cluster sharpened the broad singlet at 6·64 ppm (H_y=C–OAc, $J_{c,y}$ or $J_{x,y} < 2$ Hz) and permitted identification of H_d as a multiplet at 4·64 ppm, the multiplicity arising from spin-coupling to H_c

Table 1. NMR spectra of mikanokryptin and derivatives*

	H-1	H-2	H-6	H-7	H-8	H-9	H-10	H-13	H14†	H-15†	Misc.
3a‡	3·16 *m*§	•	5·10 *dbr* ** (6·2)	3·16 *m*	4·66 *s* (12, 10, 3·7)	•	•	6·24 *d* (3·5) 5·81 (3·1)	0·69 *d* (7·2)	1·74 *dd* (0·9, 1)	5·60 *d* (6·2, OH)
3b††	3·19 *m*§ (6·6, 6·5, 2·0, 1)	2·70 *dd* (19·4, 6·6) 2·19 *dd* (19·4, 2·0)	6·64 *br*** (w½ 4)	3·23 *m* (10, 3·5, 3·1, ~1·5)	4·64 *s* (12·4, 10, 3·7)	2·46 *dt* (12·8, 8, 3·7, 3·4) 1·95 *dt* (12·8, 12·4, 4)	2·34 *m* (7·5, 6·5, 4, 3·4)	6·35 *d* (3·5) 5·86 *d* (3·1)	0·86 *d* (7·5)	1·69 *dd* (1, 0·8)	2·06 (Ac)‖
5‡‡	3·18 *m*§§	•	6·41 *br* (w½ 3)	2·65 *m*	4·98 *ddd* (11·5, 10, 3·7)	2·5 *m* 1·9 *m*	2·1 *m*	1·27 *d* (7)‖	0·96 *d* (7·5)	1·75 *d* (1·7)	2·11 (Ac)‖
6‡‡	3·17 *m*§	•	•	2·62 *m*	4·33 *s* (12, 10, 3·7)	• 1·95 *m*	•	1·29 *d*‖ (6·7)	0·73 *d* (6·8)	1·7 *dd* (1, 0·9)	

* Values are in ppm, multiplicities are indicated by the usual symbols: *d*—doublet, *t*—triplet, *q*—quartet, *s*—septet, *m*—multiplet whose center is given, *br*—slightly broadened singlet. Unmarked signals are singlets. Values in parentheses are coupling constants or line separations.

† Three protons.

‡ In DMSO-d_6 at 90 MHz.

§ Coupled to H-15 (1 Hz).

• Obscured signal.

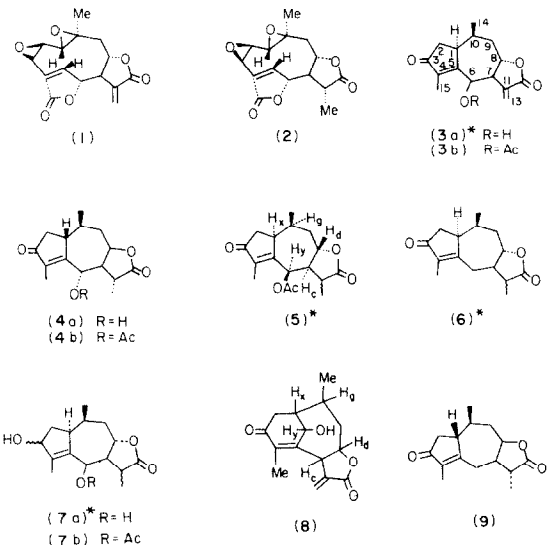
‖ Collapses to broadened singlet on addition of D_2O.

** Coupled to H-15.

†† In $CDCl_3$ at 270 MHz.

‡‡ In $CDCl_3$ at 90 MHz.

§§ Coupled to H-15 (1·7 Hz).



* Tentative configurations at C-1 and C-10.

($J_{c,d}$ = 10) as well as to two additional protons H_c and H_f ($J_{d,c}$ = 12, $J_{d,f}$ = 3·7 Hz). INDOR experiments located the signals of H_e and H_f (obviously *gem*-coupled, $J_{e,f}$ = 13 Hz) at 2·46 and 1·95 ppm; each of them was also vicinally coupled to another proton H_g whose signal appeared as a complex multiplet at 2·34 ppm. Further decoupling experiments at 270 mHz showed that H_g was responsible for splitting the methyl signal found in the NMR spectra of mikanokryptin and its derivatives (Table 1) and was also coupled to either H_c or H_x in the two-proton cluster. The former possibility was ruled out since it would have led to a four-membered ring lacking a point of attachment for the remaining carbon atoms of the molecule, thus permitting completion of A as written.

Since the NMR spectra of **3**a, **3**b and **5** exhibit no downfield signals other than those of H_a, H_b, H_d and H_y, the enone chromophore is completely substituted. One of the substituents is a methyl group (narrowly-split doublet of doublets at 1·69 ppm) which is long range coupled to H_y (J 0·9 Hz) and to either H_c or H_x in the 3·23 ppm cluster. Moreover, $CrCl_2$ reduction of **5** resulted in deoxygenation to **6** which retained the enone chromophore (cf. ref. [9] for analogous results in the geigerin series). Hence $-CH_y-OR$ is attached to the enone system as in partial structure B, and, since H_y is spin coupled to H_c or H_x, the second proton homoallylically coupled to the vinyl methyl group is either H_x or H_c.

The still missing $-CH_2-$ unit of the empirical formula could be identified in the 270 mHz spectrum of **3**b as the AB part of an ABX system where X was one of the two protons in the 3·23 ppm cluster. Combination of A and B and insertion of $-CH_2-$ between the carbonyl group and, necessarily, the carbon carrying H_x then led to two possible gross structures, **3**a and **8**. Only the former

satisfied the UV amd IR characteristics of mikanokryptin; moreover, if formula **8** were correct, it is not clear why H_x and the doubly allylic H_c should exhibit the same relatively large paramagnetic shift.

A decision between **3**a and **8** was also possible from the NMR spectrum of **5** which revealed (Table 1) that saturation of the exocyclic methylene group is accompanied by the expected upfield shift of H_c, while the frequency of H_x remains unchanged. Irradiation at the frequency of H_x did not affect the H_y signal but had some influence in the region 2–2·5 ppm (H_e, H_f and H_g). The observation that H_c but not H_x was spin coupled to H_y thus provided further grounds for eliminating **8** as a possible structure for mikanokryptin.

Its physical properties clearly differentiated acetyldihydromikanokryptin (**5**) from the previously-studied stereoisomers geigerin acetate (**4**b) and 1-epigeigerin acetate (**4**b, H-1 α) whose lactone ring is *cis*-fused; similarly, deoxydihydromikanokryptin (**6**) was different from the following five epimeric *cis*-lactones of known [9] stereochemistry, deoxygeigerin (**9**), 1-epideoxygeigerin (**9**, H-1 α), 11-epideoxygeigerin (**9**, C-11 methyl β), 1-epi-11-epideoxygeigerin (**9**, H-1 α, C-11 methyl β) and dihydroanhydrogeigerin (**9**, C-10 methyl α). This was not surprising as on the basis of Samek's rule [10] ($J_{7,13}$, *trans*-lactone $\geqq 3 \leqq J_{7,13}$, *cis*-lactone) which appears to be generally applicable to guaianolides, mikanokryptin which has $J_{7,13a} = 3{\cdot}5$, $J_{7,13b} = 3{\cdot}1$ Hz, must be a *trans*-fused γ-lactone.

The stereochemistry at two of the remaining asymmetric centers C-1, C-6 and C-10 remains speculative. The CD curves of **3**b and **5** (see Experimental) have maxima near 310 nm (n,π^* transition of cyclopentenone) and, in the case of **3**b, a second somewhat stronger maximum at 257 nm (n,π^* transition of conjugated lactone) superimposed on background curves which ascend sharply at lower wave lengths (π,π^*-transitions of ketone plus, in the case of **3**b, lactone). The positive lactone Cotton effect at 257 nm is consonant [11] with the *trans*-fusion of the lactone ring deduced in the previous paragraph if the C-7 side chain is β as in all sesquiterpene lactones of authenticated stereochemistry. The positive Cotton effect associated with the α,β-unsaturated ketone chromophore suggests that C-1 of mikanokryptin is enantiomeric with C-1 of geigerin whose ORD curve [13] exhibits a Cotton effect of opposite sign, i.e. that H-1 is α.*

Analysis of Dreiding models of mikanokryptin in which H-1 is either α or β and the lactone ring is *cis*-fused (H-7 and H-8 α) indicates that the observed coupling constants are accommodated most satisfactorily by a formula in which H-1 is α, the C-10 methyl group is β and the 7-membered ring assumes a flexible chair conformation, but regardless of the orientation at C-1 and C-10, the small value of $J_{6,7}$ requires β orientation of the hydroxyl group on C-6, as in formula **3**a. Whether H-1 be α or β, β-orientation of the hydroxyl group predicts long-range coupling between H-1 and the C-4 methyl [16] as actually observed, but only α-orientation of H-1 appears to be consonant with the additional homoallylic coupling between H-6α and H-15 found in **3**a and **3**b. On the other hand, the appearance of the vinyl methyl signal in the NMR spectrum of **5** indicates that it is homoallylically coupled to only one proton, apparently H-1.

The conversion of **5** to **6** was accompanied by a change in the sign of the Cotton effect near 310 nm, possibly due to epimerization at C-1 analogous to the formation of deoxygeigerin (**9**) from 1-epideoxygeigerin (**9**, H-1) in the presence of acid. The NMR spectrum of **6** again exhibited long-range coupling between the vinyl methyl group and two other protons, presumably H-1 and one of the two protons on H-6.

* However, the Cotton effect of α,β-unsaturated α,β-ketones of the type of mikanokryptin is affected by subtle structural alterations [14] so that this conclusion is at best tentative (see also the ORD curve of isophotosantonic acid lactone [13, 15] and some non-lactonic acid derivatives [15]. Unfortunately, neither ORD nor CD curves of 1-epigeigerin (**4**a, H-1 α) have been recorded to permit an assessment of the effect of stereoisomerism at H-1 on the cotton effect in the geigerin series.

EXPERIMENTAL

M.ps are uncorrected. Rotations were determined in $CHCl_3$ unless otherwise specified, UV spectra in 95% EtOH, IR spectra as KBr pellets, CD curves in MeOH on a Jasco Model ORD/UV recording spectrometer, mass spectra on a high resolution MS-902 mass spectrometer at 70 meV. Analyses were performed by Dr. F. Pascher, Bonn, Germany.

Extraction of Mikania micrantha. Ground herbaceous material of *Mikania micrantha* HBK, wt. 5 kg, collected by Drs. E. L. Tyson and S. McDaniel on 28 December 1969 along the Interamerican Highway 8 miles west of Chepa, Canal Zone (Tyson no. 5940, on deposit in herbarium of Florida State University) was extracted with $CHCl_3$ and worked up in the usual way [17]. The gum, wt 27 g, was chromatographed over 400 g silicic acid (Mallinckrodt 100 mesh), 500 ml fractions being col-

lected in the following order: 1–20 (C_6H_6), 21–45 (C_6H_6–$CHCl_3$, 3:1), 46–70 (C_6H_6–$CHCl_3$, 1:1), 71–100 (C_6H_6–$CHCl_3$, 1:3), 101–120 ($CHCl_3$), 121–145 ($CHCl_3$–MeOH, 97:3). Fractions 25–40 gave 1·2 g of crystalline mikanolide, fractions 50–55 gave 0·3 g of crystalline dihydromikanolide.

Extraction of 5 kg of *M. micrantha*, collected on the same date near the Pacora River Bridge 10 miles west of Chepo (Tyson no. 5941) gave only 8·7 g of crude gum. Chromatography furnished 0·3 g of mikanolide and 0·04 g of dihydromikanolide. Extraction of 13·5 g of the new species (tentative identification) collected by Dr. E. L. Tyson on 4 October 1970 6 miles east of the Rio Pacora along the Interamerican Highway (Tyson no. 6292, on deposit in herbarium of Florida State University) gave 52·5 g of crude gum. The following 500 ml fractions were collected after chromatography over silicic acid: 1–25 (C_6H_6), 25–60 (C_6H_6–$CHCl_3$, 3:1), 51–80 (C_6H_6–$CHCl_3$, 1:1), 81–100 (C_6H_6–$CHCl_3$, 1:3), 101–120 ($CHCl_3$), 121–135 ($CHCl_3$–MeOH, 99:1), 136–145 ($CHCl_3$–MeOH, 97:1). Fractions 102–115 and 122–127 eluted a gummy solid which gave a single spot on TLC. Other fractions gave complex mixtures. Recrystallization from methanol furnished 3·2 g of mikanokryptin (**3a**) m.p. 248–250°, $[\alpha]_D^{24}$ +264° (C 0·098, MeOH), UV δ_{max} 240 nm (ε 17800). IR bands at 3430, 1765, 1675 and 1632 cm^{-1}. (Calc for $C_{15}H_{18}O_4$: C, 68·69; H, 6·92; O, 24·40; MW, 262·1204. Found: C, 68·74; H, 7·02; O, 24·46; MW, 262·1206). Other significant peaks in the high resolution mass spectrum were 244·1077 (1·9%, M–H_2O) and 233·1177 (20·9%, M–CHO). Various attempts to oxidize the secondary hydroxyl group of **1a** gave complex mixtures (chromium reagents) or resulted in recovery of starting material (MnO_2).

Acetylmikanokryptin (**3b**). Acetylation of 0·3 g of **3a** with Ac_2O–pyridine, purification of the crude product by preparative TLC and recrystallization from ETOAc–hexane furnished 0·15 g of **1b**, m.p. 165–166°, $[\alpha]_D$ +340° (C 0·05). IR bands at 1760, 1735, 1685 and 1630 cm^{-1}. (Calc for $C_{17}H_{20}O_5$: C, 67·09; H, 6·62; O, 26·28. Found: C, 67·23; H, 6·98; O, 26·01).

Dihydroacetylmikanokryptin (**5**). A solution of 0·1 g of **3b** in 20 ml C_6H_6 containing 0·02 g of *tris*-(triphenylphosphine)-chlororhodium was reduced at atmospheric pressure. Hydrogen uptake ceased after absorption of one mol-eq. The solvent was removed *in vacuo*, the residue was taken up in CH_2Cl_2, filtered through florisil, evaporated and purified by preparative TLC, yield 0·06 g, m.p. 253–255°, $[\alpha]_D$ +271° (C 0·314). IR bands at 1775, 1740, 1685 and 1660 cm^{-1}. (Calc for $C_{17}H_{22}O_5$: C, 66·65; H, 7·24; O, 26·11; MW 306·1466. Found: C, 66·32; H, 7·39; O, 25·89; MW, 306·1474). Other significant peaks in the mass spectrum were 264·1361 (base peak, M–C_2H_2O), 246·1259 (24·1%, M–$C_2H_4O_2$) and 218·1307 (17·5%, M–$C_2H_4O_2$–CO).

Deoxydihydromikanokryptin (**6**). A solution of 0·06 g of 5 in 6 ml EtOAc and 1 ml of HOAc was stirred with a 1 M $CrCl_3$ solution, freshly prepared from Zn amalgam and $CrCl_5$, at room temp. for 2 days, diluted with H_2O and thoroughly extracted with Et_2O. The washed and dried Et_2O extracts were evaporated and the residue purified by preparative TLC. The major fraction, **6**, was recrystallized from EtOAc–hexane, yield 0·025 g, m.p. 149–151°, IR bands at 1770, 1685 and 1635 cm^{-1}. [Calc for $C_{15}H_{20}O_3$: C, 72·55; H, 8·12; O, 19·22; MW, 248·1412. Found: C, 71·90; H, 7·89; O, 19·68; MW, 248·1415 (base peak).]

$NaBH_4$ Reduction of **3a** *and* **3b**. A solution of 50 mg of **3a** in 40 ml of MeOH was stirred with 40 mg of $NaBH_4$ at 0° for 3 hr. The acidified (dil. acetic acid) solution was taken to dryness, H_2O was added and the organic material extracted with EtOAc. The dried extract was evaporated and the residue (**7a**) recrystallized from ethyl acetate–hexane, yield 30 mg, m.p. 194–196°, IR bands (KBr) at 3350, 3320 and 1750 cm^{-1}. The NMR spectrum (pyridine-d_5) exhibited signals for two secondary hydroxyl protons at 6·70 *m* (H-3) and 6·37 *br* (H-6) and a new secondary methyl group at 1·37 ppm (*d*, *J* 6·5 Hz). Reduction of 50 mg of **3b** in a similar manner afforded, after recrystallization from EtOAc–hexane, 30 mg of **7b**, m.p. 189–191°.

CD and ORD curves (0·4 *mg/ml in MeOH*). Mikanokryptin (**3a**): $[\theta]_{360}$ 0; $[\theta]_{335}$ 1365; $[\theta]_{310}$ 3460 (max); $[\theta]_{265}$ 0 (last reading). Acetylmikanokryptin (**3b**): $[\theta]_{375}$ 0; $[\theta]_{340}$ 625; $[\theta]_{313}$ 1500 (max); $[\theta]_{300}$ + 1250; $[\theta]_{280}$ 690 (min); $[\theta]_{257}$ + 2190 (max), $[\theta]_{253}$ 1500 (min); $[\theta]_{231}$ 31300 (max); $[\theta]_{225}$ 23100; $[\theta]_{210}$ +37600 (last reading). ORD $[\psi]_{350}$ 4800; $[\psi]_{275}$ 9120; $[\psi]_{245}$ +27400 (max); $[\psi]_{235}$ +19800; $[\psi]_{225}$ 0; $[\psi]$ −15200 (last reading). Dihydroacetylmikanokryptin (**5**): $[\theta]_{375}$ 0; $[\theta]_{325}$ 615; $[\theta]_{313}$ 756 (max); $[\theta]_{290}$ 370; $[\theta]_{270}$ 74 (min); $[\theta]_{257}$ 1350 (last reading). ORD $[\psi]_{450}$ 370; $[\psi]_{400}$ 500; $[\psi]_{370}$ 600; $[\psi]_{330}$ 870; $[\psi]$ 920 (sh); $[\psi]_{280}$ 1800; $[\psi]_{260}$ 4000 (last reading). Deoxydihydromikanokryptin (**6**): $[\theta]_{350}$ 0; $[\theta]_{325}$ −1100; $[\theta]_{306}$ −2000 (min); $[\theta]_{275}$ −660 (weak sh); $[\theta]_{268}$ 0; $[\theta]_{240}$ 23200 (max); $[\theta]_{220}$ 11800; $[\theta]_{210}$ 5400 (last reading). ORD $[\psi]_{325}$ −3000; $[\psi]_{300}$ 0; $[\psi]_{275}$ 4200; $[\psi]_{250}$ 20600 (max); $[\psi]_{232}$ 0; $[\psi]_{225}$ −12100; $[\psi]_{220}$ −32700 (last reading).

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