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THE STRUCTURE AND CHEMISTRY OF ISABELIN

A NEW GERMACRANOLIDE DILACTONE FROM *AMBROSIA PSILOSTACHYA* DC. (COMPOSITAE)[1]

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Abstract—Details are given for the isolation, structure determination and chemistry of isabelin (**1**), a new germacranolide dilactone from *Ambrosia psilostachya*. NMR studies at different temperatures established that isabelin exists in solution at room temperature in a 10 :7 ratio of two conformers.

The dihydro and hexahydro derivatives of isabelin were key compounds for the structure elucidation since these derivatives were correlated by IR with known substances. In the course of the structure analysis, a number of other derivatives and transformation products were prepared and characterized; these included tetrahydroisabelin, mono- and dipyrazolines, Michael adducts, isoisabelin, bromodihydroisoisabelin and two ketocarboxylic acid methyl esters.

A separate proof for the structure of isabelin was provided by its synthesis from cnicin (**5**), a germacranolide from *Centaurea* species.

INTRODUCTION

THE species *Ambrosia psilostachya* DC. (Compositae) is remarkable for the number and types of sesquiterpene lactones which it elaborates:[2] the pseudoguaianolides: coronopilin, parthenin, ambrosin, damsin, 3-hydroxydamsin, cumanin, cumanin 3-acetate, cumanin diacetate, and ambrosiol, and the cleaved pseudoguaianolides: psilostachyin, psilostachyin B and psilostachyin C. In a continuation of our biochemical systematic studies of this species, we now report the isolation and properties of the first germacranolide-type sesquiterpene lactone from this species. The material exhibited an unusual NMR spectrum which displayed signals for what appeared to be a 10 :7 mixture of two compounds. Nevertheless, the material behaved as a single compound chromatographically and chemically. We have now established that the new germacranolide, which we named isabelin, exists in solution at room temperature as a 10 :7 ratio of two conformers. In this paper we describe the details for the isolation, structure determination and transformations of isabelin.

Isolation and physical properties of isabelin (**1**)

We isolated isabelin first from *Ambrosia psilostachya* plant material collected near Alice, Texas in 1965, and later from material obtained from Port Isabel, Texas; the compound is named for the latter collection site. A 1968 collection from Port Isabel yielded isabelin mixed with smaller amounts of psilostachyin and psilostachyin B.

Isabelin (**1**), $C_{15}H_{16}O_4$, m.p. 169–170°*, $[\alpha]_D^{25}$ −57·2° ($CHCl_3$; *c*, 0·87), exhibited

* Isabelin quickly polymerizes at temperatures above 100°; therefore a sharp m.p. was obtained only by placing crystals on a hot plate already heated to about 168°.

UV and IR spectra typical for the presence of α,β-unsaturated γ-lactones: UV, λ_{max} 211 nm, ε 18 900 [for model compounds see the dilactone germacranolides elephantopin[3] (λ_{max} 215 nm, ε 25 200) and mikanolide[4] (λ_{max} 206 nm, ε 17 600); IR, strong band at 1750 cm^{-1} characteristic for α,β-unsaturated γ-lactones and weak double bond bands at 1640 and 1660 cm^{-1}.

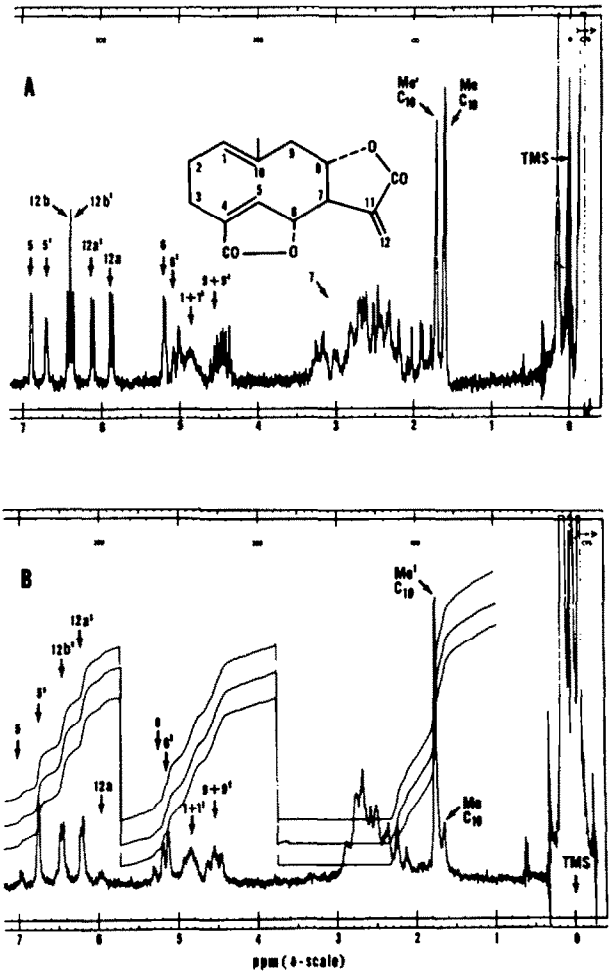


FIG. 1 The 100 Mc NMR spectra of isabelin: (A) in $CDCl_3$ at 25°; and (B) in $CDCl_3$ which had been precooled to −50° before the crystals were dissolved. In spectrum A, the signals for the major conformer are designated with unprimed numbers; however at −50° (spectrum B), this conformer appears to be the minor constituent.

The most remarkable spectral property of isabelin was the NMR spectrum recorded in $CDCl_3$ at 25° (Fig. 1A). The spectrum exhibited two sets of signals which appeared to correspond to two compounds in a 10:7 ratio. This interpretation gained support from the following temperature studies: When the $CDCl_3$ solution of isabelin was cooled to −60° and the NMR spectrum was recorded at that temperature essentially

the same 10:7 ratio was observed. Moreover, the same results were obtained when a spectrum was determined at 70° in $^2H_6$-DMSO. Because isabelin rapidly polymerizes at temperatures above 100°, higher temperature NMR studies were not possible.

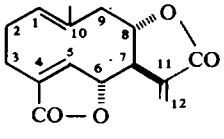
Nevertheless, the material behaved as a pure compound chromatographically (TLC analysis in 25 solvent systems), during fractional crystallization experiments (six solvent systems) and, as we shall see later, during the preparation of a number of derivatives and transformation products. These results suggested that the NMR spectrum of isabelin (Fig. 1A) should be interpreted on the basis of isabelin existing in solution at room temperature as two conformational isomers. Conclusive evidence for the latter interpretation was provided by the following NMR study: Crystalline isabelin was dissolved in $CDCl_3$ precooled to $-50°$ and an NMR spectrum was recorded at that temperature within 40 minutes (Fig. 1B). At $-50°$, the major isomer appeared to correspond to the minor form at 25°,* which may be the only conformer present in the crystals.† In this connection, it is of interest that an NMR spectrum taken within 10 seconds after dissolving isabelin in $CDCl_3$ at 25° already showed the 10:7 ratio of conformers.

The NMR signal assignments for isabelin noted in Fig. 1A and recorded in Table 1 are in accord with published interpretations for other related sesquiterpene lactones[3,4] and are based in part upon the spin-decoupling experiments described below. The principle feature of the NMR spectrum shown in Fig. 1A are the following pairs of signals which are present in a 10:7 ratio: a broadened singlet for a conjugated olefinic C-5 proton at 6·90‡ and one at 6·76; a doublet at 5·88 (J = 3) for one of the exocyclic protons at C-12 in the major conformer and a similar signal at 6·14 (d, J = 3) for a C-12 proton in the minor conformer; and vinylic C-10 methyl singlets in a 10:7 ratio at 1·60 and 1·68.

A pair of signals in a 10:7 ratio (5·03, dd, J = 2 and 7; 5·25, complex signal) were typical for a lactone proton signal and were ascribed to the proton at C-6; spin decoupling experiments established that these signals were coupled to the signals for the C-5 olefinic protons in the two conformers.

The C-12 exocyclic proton doublets (major conformer: 5·88 and 6·42; minor conformer: 6·14 and 6·38) were collapsed into singlets by irradiation at 3·24 and 2·65, respectively, the regions where H-7 proton signals are known to occur. Irradiation at either 3·24 or 2·65 also altered the multiplet at 4·50, a signal in accord with a lactonic proton at C-8.

Although differences in the signal patterns were observed when the NMR spectrum



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* When the solution used for this $-50°$ NMR study was allowed to warm to room temperature, it showed by NMR the 10:7 ratio of conformers.

† Attempts to determine the crystalline structure of isabelin by X-ray crystallography were unsuccessful because isabelin decomposed upon X-ray irradiation.

‡ All chemical shift values are reported in ppm (δ scale); s = singlet; d = doublet; dd = double doublet; tr = triplet; m = multiplet and c = complex signal.

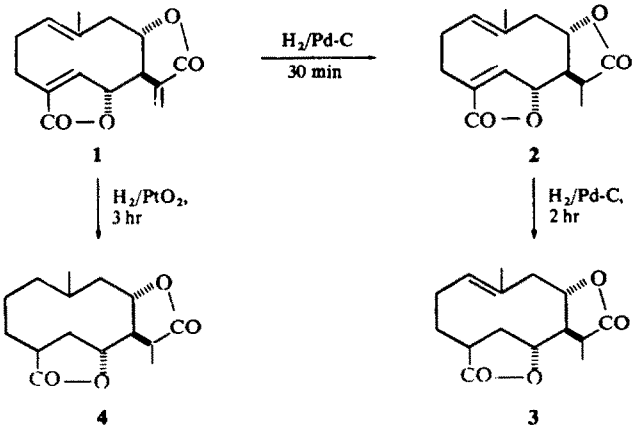
of isabelin was recorded at room temperature using different solvents, in all solvent systems employed ($CDCl_3$, CH_3CN, $^2H_5$-pyridine, CF_3COOH and dimethylformamide) isabelin existed as a 10 :7 ratio of two conformers (Table 1).

When the spectral results were considered together with the structure of other germacranolides known to be elaborated by species belonging to the family Compositae,[5,6] structure **1** was one of only a few reasonable possibilities for isabelin. That structure **1** did indeed represent isabelin was established by: (1) preparing the di and hexahydro derivatives which were identical by IR spectra with known compounds, and (2) by synthesizing isabelin from cnicin (**5**), a known germacranolide.

Hydrogenation of isabelin

The first proof that the isabelin material represented a single substance belonging to the germacranolide dilactone series of sesquiterpenes resulted from the analysis of the hydrogenation products.

Hydrogenation of isabelin for 30 minutes with 5% Pd-C as catalyst afforded dihydroisabelin (**2**), $C_{15}H_{18}O_4$, m.p. 184°, in 95% yield. The UV (λ_{max} 209 nm, ε 9400), IR (1740 cm^{-1} carbonyl and 1644 cm^{-1}, double bonds), and especially the NMR spectrum (loss of the signals for the C-12 exocyclic double bond protons and the appearance of a new 3-proton doublet ($J = 7$) at 1·44, typical for a new C-11 Me group) were in accord with structure **2**. Significantly, the NMR spectrum indicated that isabelin had afforded in almost quantitative yield a compound which essentially existed as a single conformer,* thus supporting the view that the isabelin material represented a single substance. The IR spectra of dihydroisabelin (m.p. 184°) and germacrenediolide† (previously assigned structure **2**)[8] were identical. Unfortunately an authentic sample of germacrenediolide was not available and since its reported[8] m.p. was 10° lower than that observed for dihydroisabelin, the IR correlation was not considered conclusive.



* The NMR spectrum and gas chromatographic analysis of dihydroisablin did show a 10:1 ratio of two compounds. However, because it has been our experience[7] that hydrogenations of the exocyclic double bonds in sequiterpene lactones with Pd-C as catalyst usually yields a mixture of the C-11 epimers in various ratios, we assume here that the 10:1 ratio represents the C-11 epimeric forms of dihydroisabelin.

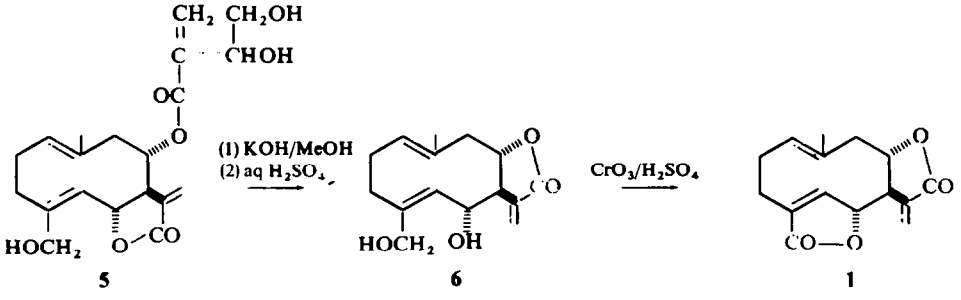
† We thank Dr. V. Herout, Czechoslovak Academy of Science, Prague, for the IR spectra of germacrenediolide **2** and germacranediolide, which was shown to correspond to hexahydroisoabelin (**4**).

Dihydroisabelin slowly absorbed a second mole of hydrogen in the presence of the Pd-C catalyst to give a tetrahydro derivative, $C_{15}H_{20}O_4$, m.p. 148°, to which we assign structure **3** on the basis of the UV, IR and NMR spectral data: For example, the UV spectrum of **3** did not exhibit the high intensity peak expected for an α,β-unsaturated γ-lactone but instead showed a weak max at 210 nm (ε 2950), and the NMR spectrum did not display a conjugated olefinic proton signal but did show a 3-proton vinylic Me group signal at 1·70.

When isabelin was exhaustively hydrogenated in aqueous acetic acid with PtO_2 as catalyst, a hexahydro derivative, $C_{15}H_{22}O_4$ m.p. 193–194°, was obtained. The IR spectrum of hexahydroisabelin was identical with that of germacranediolide (**4**)*, a substance derived from cnicin† (**5**).[8] Moreover, since the m.p. reported for germacranediolide corresponded to that observed for hexahydroisabelin it is reasonable to assign structure **4** to hexahydroisabelin and thus structure **1** to isabelin.

Synthesis of isabelin from cnicin (**5**)

The final proof of structure **1** for isabelin was provided by its synthesis from cnicin: Cnicin was hydrolyzed with methanolic potassium hydroxide to yield upon acidification with dilute sulfuric acid‡ a diol which corresponded to artemisiifolin (**6**), a new germacranolide which we recently encountered in *Ambrosia artemisiifolia* L.[11] Oxidation of the diol **6** in acetone with chromium oxide-sulfuric acid[12] afforded material identical in all respects with isabelin. It is noteworthy that in contrast to isabelin both cnicin (**5**) and artemisiifolin (**6**) appear to exist as single conformers in solution at room temperature on the basis of their NMR spectra.



5 **6** **1**

The conformational forms of isabelin

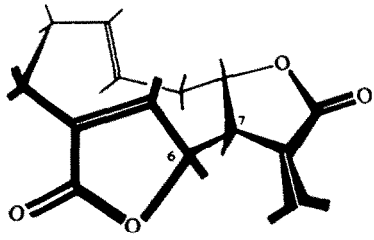
Molecular models indicated that two conformational forms of isabelin involving rotation of the C_1-C_{10} trisubstituted vinyl group around the C_1-C_2 and C_9-C_{10} bonds are preferred. On the basis of the following arguments we assign the sterically preferred structures (A) and (B), respectively, to the two conformers of isabelin present

* See second footnote on previous page.

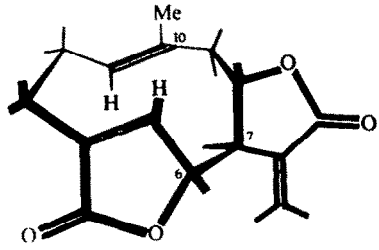
† The absolute configuration at C-6, C-7 and C-8 in cnicin were recently revised as shown in structure **5**[9]. Moreover, recent work by Z. Samek, M. Holub, V. Herout and F. Sorm, Tetrahedron Letters (in press), indicate that the acyl moiety of cnicin has the structural features shown in formula **5** and is attached at C-8 rather than C-6.

‡ We (unpublished) and others[10] have independently established that although the hydrolysis conditions open the lactone ring, relactonization in germacranolides of this type occurs to C-8 rather than C-6.

in solution at room temperature in a 10:7 ratio: The bond angle between H_7 and H_6 in conformer (A) is approximately 90°, a value in accord with a small coupling constant:



(A)



(B)

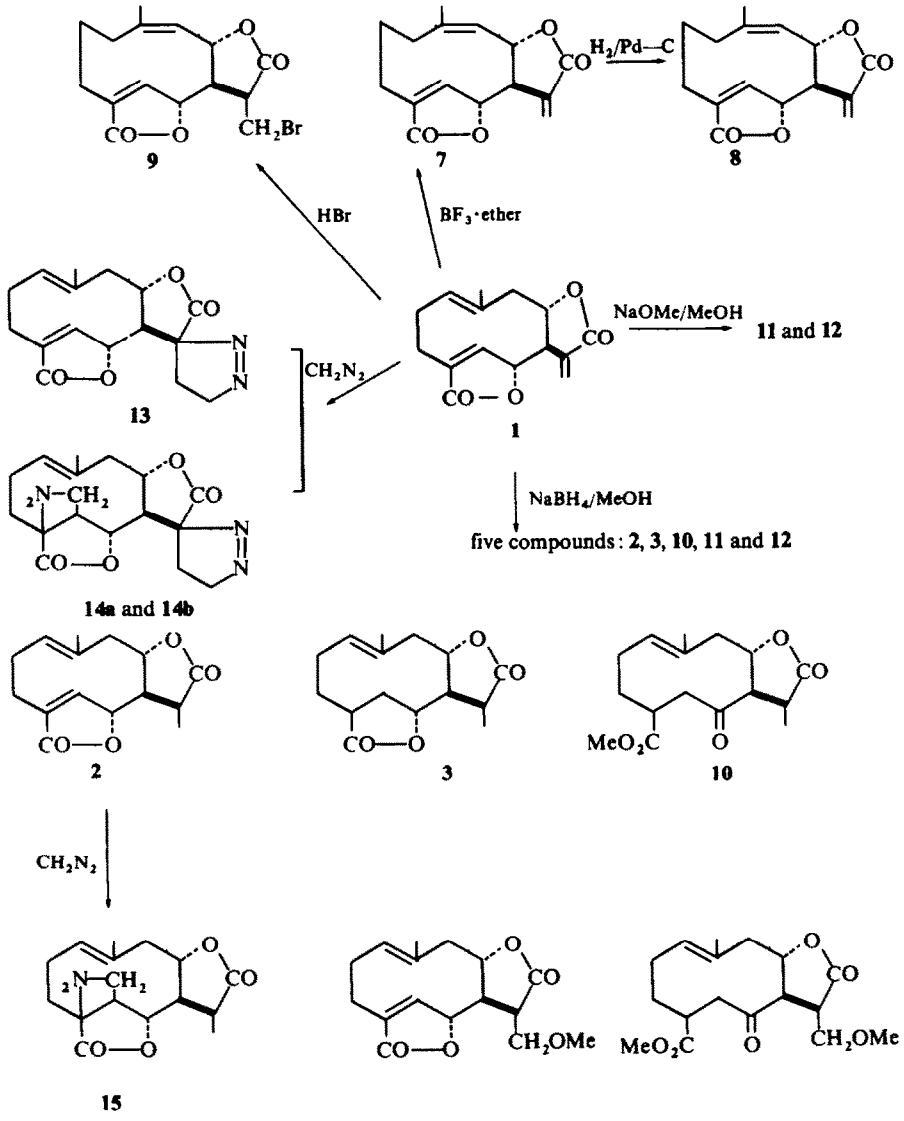
the major form of isabelin present in solution at room temperature showed a 1 c/s coupling between H_7 and H_6 and is therefore assigned structure (A). The bond angle between H_7 and H_6 in conformer (B) is approximately 180° and thus a large coupling constant is expected; the NMR data (including spin decoupling results) established that the minor conformer of isabelin exhibited a 7 c/s coupling constant between the protons in question and thus can be assigned structure (B).

The downfield position (δ, 6·14) for the signal which corresponds to the proton at C_{12} which is *trans* to the C_{11} carbonyl function in the minor conformer can be rationalized on the basis of structure (B): In (B) the trans C_{12}-proton is deshielded as a result of the diamagnetic anisotropic effect of the second lactone function.

Derivatives and transformation products derived from isabelin

A number of interesting dérivatives and transformation products were prepared in the course of the structure investigations. The properties and preparations of these compounds are recorded in Table 1 and in the experimental section and only briefly mentioned here.

Isoisabelin (7). In the presence of boron trifluoride etherate, isabelin was slowly but quantitatively transformed into an isomeric substance, which we named isoisabelin and to which we assign structure 7. Isoisabelin (λ_{max} 209 nm, ε 13 500) exhibited an NMR spectrum corresponding to a single conformer; the spectrum could best be interpreted on the basis of migration of the C_1—C_{10} double bond to C_9—C_{10} (Table 1). Moreover, correlation of the NMR data (in particular, a 2 c/s coupling constant between H_7 and H_6) with molecular models suggested that isoisabelin (7)



and its dihydro derivative (**8**) have a *cis* configuration about the C_9—C_{10} double bond. Treatment of isabelin with hydrogen bromide produced a bromo derivative to which we assign structure **9**, which also contains a *cis* C_9—C_{10} double bond.

Michael adducts and rearranged products. Treatment of isabelin with methanolic sodium borohydride yielded a mixture of five compounds: di- and tetra-hydroisabelin (**2** and **3**, respectively), and the 11,12-dihydro rearranged product **10**, the Michael adduct **11** and the rearranged Michael adduct **12**. The latter two compounds, **11** and **12**, were also obtained when isabelin was treated with dilute methanolic sodium methoxide: **11** after 30 min and **12** after standing overnight. In addition, dihydroisabelin (**2**) gave the rearranged product **10** upon treatment with dilute methanolic

TABLE 1. NMR DATA OF ISABELIN AND RELATED COMPOUNDS

		H_1	H_9	H_8	H_7	H_6	H_5	H_{11}	H_{12}	C_4-Me	C_{11}-Me	Other
1[b]	(A)	4·88c		4·48c	3·22c	5·12c	6·90c		5·88d (3) 6·42d (3)	1·60d (2)		
	(B)				2·65[c]c	5·06dd (7·2)	6·70c		6·14d (3) 6·33d (3)	1·68d (2)		
1[d]	(A)				3·30c	5·36c	7·17c		5·87d (3) 5·98d (3)			
	(B)					5·15 br.d (6)	6·87c		6·35t (3)	1·68d (2)		
2[b]		4·88c		4·40m	3·08c	5·04c	6·92c 6·71c			1·61d 1·68(2)	1·44d (7)	
3[b]		5·30c		4·60c	3·08c	4·60c		2·30[e]		1·70d (2)	1·30d (7)	
4				4·72c		4·72c				0·87d (6)	1·28d (6)	
7[b]			5·08 br.d (11)	4·85t (10)	3·14c	5·34c	7·05c		5·65d 6·33 (4)	1·76d (2)		

8[b]		5·05c	4·82t (10)	2·65c	4·91c	7·20c	2·45c		1·74d (2)	1·37d (6)	
9[f]		4·8	5·3	3·21c	7·50c			3·78c	1·80c		
10	5·33 (br.t 7)	2·67dd (8, 4)	4·50m	2·76c		2·73			1·53d (2)	1·26d (7)	CH_3-ester 3·65 S
11	4·84c		4·36m		5·22c	6·82c		3·75c	1·61d (2)		CH_3 ether 3·385
12	5·42 br.t (7)	2·75dd (12, 4)	4·85dq (12, 10, 4)	3·08d (10)		2·92c		3·64c	1·57d (2)		CH_3-ester 3·68 CH_3 ether 3·34
13	5·05				4·40c	6·86c			1·60c		
14a									1·64c		3·388d (5) 3·52d (10)
14b									1·64c		3·86d (5)
15	5·36c						2·32[c]		1·60c	1·15d (6)	4·23d (5)

[a] Spectra were determined in $CDCl_3$ on a Varian A-60 spectrometer unless otherwise noted. Values are given in parts per million relative to tetramethylsilane as an internal standard. Numbers in parentheses denote coupling constants in c/s. Singlets are unmarked. Multiplets are described as follows: d = doublet, dd = double doublet; t = triplet, q = quartet, m = multiplet, c = complex signal whose center or range is given.

[b] Spectrum recorded on Varian HA-100 spectrometer.

[c] This chemical shift was only determined by spin decoupling experiments.

[d] Spectrum recorded in $^2H_5$—C_5H_5N.

[e] Spectrum recorded in $^2H_6$—DMSO.

[f] Spectrum recorded in CF_3COOH.

sodium borohydride. The structure assignments for all of these compounds are based primarily upon the IR, UV and NMR spectral results recorded in Table 1 and in the experimental section.

Pyrazolines. Treatment of isabelin with diazomethane afforded one mono- and two di-pyrazoline derivatives to which we assign structures **13** and **14** (a and b), respectively, on the basis of the available spectral data (see Table 1 and Experimental). Dihydroisabelin (**2**) also was converted to a monopyrazoline derivative to which we assign structure **15**.

EXPERIMENTAL*

Isolation of isabelin. A small collection of *Ambrosia psilostachya* DC. was made six miles north of Alice, Texas, Oct. 17, 1965 (Voucher No. 342653)† and larger collections were obtained in Port Isabel, Texas, May 4 and 5, 1967 (Voucher Nos. 255657 and 255644). The air-dried plant material (leaves and stems) was extracted with chloroform and worked up by the usual procedure.[13] The syrup obtained from 600 g of plant material afforded 6·2 g crystalline isabelin; no other sesquiterpene lactones were detected.‡

Recrystallization of the crude material from EtOAc afforded pure **1** m.p. 169–170° (on a hot plate); $[\alpha]_D^{25}$ −57·2°; ($CHCl_3$ *c*, 0·87); UV: λ_{max} (C_2H_5OH): 211 nm (ε 18 900); IR bands (Nujol): 1750 (carbonyls), 1660 and 1644 (double bonds), 910, 877, 860, and 810 cm^{-1}; R_f 0·63 (silica gel G, $CHCl_3$–ether, 5:1); [Found: C, 69·36; H, 6·27; O, 24·50. Mol. wt. (mass spec.) 260. $C_{15}H_{16}O_4$ requires: C, 69·21; H, 6·20; O, 24·59%].

Dihydroisabelin (**2**). Isabelin (**1**; 385 mg) was hydrogenated for 30 min in 40 ml of a 1:1 soln of EtOAc and EtOH in the presence of 5% Pd-C (80 mg) which had been prehydrogenated for 15 min.

Recrystallization of the crude material obtained upon workup of the reaction mixture from 95% aqu EtOH (3 ml) afforded small prisms (275 mg). Concentration of the mother liquor to 1 ml yielded an additional 23 mg. The viscous oil (80 mg) which remained after evaporation of the final mother liquor was separated by thick-layer TLC (silica gel G; benzene-Me_2CO-EtOH, 50:6:1·5). The major band (R_f value 0·27–0·50), which was extracted with Me_2CO, afforded a colorless oil (68 mg) which slowly crystallized, giving **2**, m.p. 182–184°, $[\alpha]_D^{25}$ ±0°, $[\alpha]_{365}^{25}$ −182° ($CHCl_3$, *c*, 0·17); UV (EtOH): λ_{max} 209 nm (ε 9400); IR bands ($CHCl_3$): 1740 (carbonyls) 1644 (double bonds), 1172, 1006, 990, 862, and 848 cm^{-1}; R_f 0·47 (silica gel G, benzene-Me_2CO-EtOH 50:6:1·5). [Found: C, 68·65; H, 6·87; O, 24·40. $C_{15}H_{18}O_4$ requires: C, 68·68; H, 6·87; O, 24·40%]. The IR spectrum of dihydroisabelin was identical with that of germacrenediolide, a substance derived from **5**.

Tetrahydroisabelin (**3**). Isabelin (**1**; 265 mg) was hydrogenated for 2 hr as described above. Recrystallization of the crude product from EtOH (4 ml) afforded feather-like needles (90 mg) mixed with a few prisms. Concentration of the mother liquor to about 2 ml gave only needles.

Both the prisms and needles corresponded to **3**, m.p. 148°, $[\alpha]_D^{25}$ ±0°; $[\alpha]_{365}^{25}$ −65° ($CHCl_3$, *c*, 0·4); UV (C_2H_5OH): λ_{max} 208 nm (ε 2950); IR bands (Nujol): 1740 (carbonyls), 1200, 1180, 980 and 768 cm^{-1}; R_f 0·41, (silica gel G; $CHCl_3$–ether, 5:1). [Found: C, 68·05; H, 7·75; O, 24·23. $C_{15}H_{20}O_4$ requires: C, 68·03; H, 7·58; O, 24·22%].

Hexahydroisabelin (**4**). Isabelin (250 mg) was hydrogenated for 3 hr in 36% aqueous HOAc (27 ml) in the presence of 100 mg PtO_2 which had been prehydrogenated for 20 min. Recrystallization of the crude product from EtOH (2 ml) gave long plates (120 mg), m.p. 191–193°. Further recrystallization from EtOH afforded pure **4** as long plates, m.p. 193–194°, UV (EtOH); λ_{max}, 209 nm (ε 363); IR bands ($CHCl_3$): 1745 (carbonyls), 1175, 1010 and 977 cm^{-1}; R_f 0·42 (same system as above). [Found: C, 67·48; H, 8·15; O, 24·05. $C_{15}H_{22}O_4$ requires: C, 67·64; H, 8·33; O, 24·05%]. The IR spectrum of **4** was identical with that of germacranediolide, a substance derived from **5**.

Synthesis of isabelin (**1**) *from cnicin* (**5**). A soln of **5** (1·2 g) in 25 ml MeOH was treated 1 hr on a steam bath with occasional shaking with methanolic KOH (3 g in 25 ml). The hydrolysis soln was taken to dryness and the residue was dissolved in cold H_2O (30 ml). The aqueous soln was acidified with 5% H_2SO_4 (30 ml). The

* All m.ps are uncorrected. Analyses were determined by Dr. Alfred Bernhardt, Mikroanalytisches Laboratorium· Elbach über Engelskirchen, West Germany.

† All vouchers are depositied in The University of Texas Herbarium, Austin.

‡ Collections obtained in Port Isabel in 1968 (J. Landis No. 25) did contain some psilostachyin and psilostachyin B along with the major component, isabelin.

soln was shaken with ether (50 ml) and filtered. The residue was washed with additional ether; the ether layer in the filtrate was separated· washed with $NaHCO_3$aq, NaClaq and dried over anhyd Na_2SO_4.

Evaporation of the ether left a colorless viscous oil which was separated by preparative TLC (silica gel G, benzene-$CHCl_3$-Me_2CO, 1:1:1). The major band yielded a crystalline compound. Recrystallization of the crude material from EtOAc afforded crystals (130 mg) m.p. 128–130°, which were identical with authentic **6**[11] by m.mp., TLC, IR and NMR.

The diol **6** was dissolved in acetone (50 mg in 2 ml); the soln was cooled and maintained below 35° during the addition of 15 drops of a solution of CrO_3 in H_2SO_4.[12] The soln was shaken vigorously during the addition of the oxidizing reagent. After standing for 1 min the reaction mixture was diluted with 10 ml of cold H_2O and then extracted with two 10 ml portions $CHCl_3$. The $CHCl_3$ layer was washed with H_2O and $NaHCO_3$aq and then dried over anhyd Na_2SO_4. Preparative TLC separation of the crude material obtained from the $CHCl_3$ extract (silica gel G; benzene-acetone, 4:1) gave 15 mg of material which was identical in all respects with the natural **1**.

Isoisabelin (**7**). BF_3-Et_2O (3 ml) was added to a soln of isabelin (500 mg) in $CHCl_3$ (15 ml). The reaction flask was then equipped with a $CaCl_2$ drying tube and placed in the dark at room temp for 72 hr. The reaction solution was discharged into 150 ml cold H_2O and neutralized with $NaHCO_3$aq. A $CHCl_3$ extract upon workup afforded a yellow powder (490 mg).

Recrystallization of the powder from $CHCl_3$ (5 ml) gave **7** (150 mg) as small prisms. When the mother liquor was concentrated to 2·5 ml another 30 mg **7** was obtained. TLC and NMR analysis of the oil (300 mg) remaining from the final mother liquor indicated a 1:1 mixture of **1** and **7**. Isoisabelin (**7**) sintered at 237° and decomposed at about 255°; $[\alpha]_D^{25}$ +74·0° ($CHCl_3$, *c*, 0·35), R_f 0·55 (silica gel G; $CHCl_3$-EtOH, 5:0·1); UV (EtOH): λ_{max} 207 nm (ε 13400); IR bands (Nujol): 1750 (carbonyls), 1650 (double bonds), 1138, 884, 815 and 790 cm^{-1}. [Found: C, 69·24; H, 6·22; O, 24·77. $C_{15}H_{16}O_4$ requires: C, 69·21; H, 6·20; O, 24·59%].

Isoisabelin (50 mg) yielded a dihydro derivative when hydrogenated as described above for the preparation of dihydroisabelin. Recrystallization of the crude hydrogenated material from 2 ml of EtOAc-EtOH, 1:1, afforded **8** as long plates (35 mg), m.p. 248°; IR bands (Nujol): 1770 and 1747 (carbonyls), 1650 (double bonds), 1032, 975, 910, 872 and 817 cm^{-1}.

12-*Bromo*-11,12-*dihydro-isoisabelin* (**9**). Anhyd HBr was bubbled slowly for 2 hr into a soln of isabelin (50 mg) in benzene (15 ml) at room temp. (The soln became saturated with HBr during the first minutes of the 2 hr reaction period). Excess HBr was subsequently removed with a stream of dry N_2 (1 hr).

Colorless needles which precipitated during the removal of excess HBr were filtered and washed with 5 ml benzene (43 mg); R_f 0·58, TLC (silica gel G, $CHCl_3$–ether, 5:1). The crystals contained about $\frac{1}{3}$ mole equivalent of benzene (by NMR). Recrystallization of the benzene-solvated material from EtOAc afforded pure **9**; m.p. 224–224·5° (on a hot plate); IR bands (Nujol): 1780 and 1748 (carbonyls, 970, 910, 845, 797 and 674 cm^{-1} (C-Br). [Found: C, 52·80; H, 5·18; O, 18·85; Br, 23·31. $C_{15}H_{17}O_4Br$ requires: C, 52·68; H, 4·99; O, 18·75; Br, 23·41%].

Michael adduct (**11**) *and rearranged Michael adduct* (**12**) *from isabelin*. Isabelin (333 mg) in MeOH (10 ml) was mixed with methanolic NaOMe prepared from Na (6 mg) and MeOH (0·5 ml). After 30 min the soln became transparent and it was then poured into cold H_2O (50 ml) and acidified with 1N HCl (2 ml). The acidic soln was extracted with 3 × 20 ml portions $CHCl_3$. The $CHCl_3$ yielded upon workup and TLC separation (silica gel G, $CHCl_3$–ether, 5:1), a colorless oil (280 mg) which crystallized on standing. Recrystallization of the material from CCl_4 (5 ml) afforded needles (200 mg), which contained a mole equivalent of CCl_4. The CCl_4 could be removed by warming the crystals on a steam bath *in vacuo* for 6 hr. The CCl_4-free Michael adduct (**11**) had the following properties: m.p. 118°; IR bands (Nujol): 1750, 1725 (carbonyls), 1100, 1010, 975 and 870 cm^{-1}. [Found: C, 65·67; H, 6·90; O, 27·55. $C_{16}H_{20}O_5$ requires: C, 65·73; H, 6·95; O, 27·40%].

When a similar reaction soln using 265 mg of isabelin was allowed to stand overnight and then worked up a rearranged **12** was obtained [102 mg after recrystallization of the crude material (150 mg) from 2 ml of n-hexane containing 5% ether]; m.p. 99·5–100·5°; $[\alpha]_D^{25}$ +36·8 (EtOH, *c*, 0·50); UV (EtOH): λ_{max} 277 nm (ε 25) (ketone group) and λ_{max} 212 nm (ε 2720); IR bands (Nujol): 1750 (lactone), 1720 (ester), 1700 (ketone), 1196, 1000 and 845 cm^{-1}. [Found: C, 63·15; H, 7·39; O, 29·81. $C_{17}H_{24}O_6$ requires: C, 63·15; H, 7·14; O, 29·66%].

A rearranged product (**10**) *from dihydroisabelin* (**2**). When dihydroisabelin (274 mg) in 10 ml MeOH was treated with NaOMe (from 12 mg Na and 6 ml MeOH) for 3 hr and worked up as described above, 300 mg of crude crystalline material was obtained.

Recrystallization of the crude material from CCl_4 (5 ml) afforded small prisms (255 mg) of **10**, m.p. 147–

149°; IR bands (Nujol): 1752 (lactone), 1730 (ester), 1680 (ketone), 1192, 1000 and 845 cm^{-1}. [Found: C, 65·22; H, 7·41; O, 27·34. $C_{16}H_{22}O_5$ requires: C, 65·30; H, 7·48; O, 27·20%].

Products from the treatment of isabelin with sodium borohydride

Dihydro-(2) and tetrahydro-isabelin (3), rearranged dihydroisabelin (10), Michael adduct (11) and rearranged Michael adduct (12). (A) Isabelin (260 mg) in 5 ml of MeOH was mixed with stirring and cooling with 50 mg of $NaBH_4$ dissolved in 5 ml of MeOH. The addition required about 12 min and after an additional 2 to 3 min the soln was mixed with 50 ml of H_2O containing 1 ml 6N HCl. $CHCl_3$ extraction and workup afforded 200 mg of an oil which consisted essentially (by NMR) of a 2:1 mixture of **2** and **3**. The crude mixture (40 mg) was separated by TLC (silica gel G, benzene-Me_2CO-EtOH, 50:6:1·5); the band at R_f 0·47 gave 8 mg of pure **2**, m.p. 183°, while the band at R_f 0·41 yielded 15 mg of pure **3**, m.p. 147°.

When isabelin (300 mg) was treated overnight with $NaBH_4$ (300 mg in 6 ml of MeOH), the following compounds were isolated after TLC separation: Adduct **11**, 90 mg; and 30 mg of a 2:1 mixture of the rearranged **10** and **12** as co-crystals, m.p. 102–103°; **10** and **12** were identified by NMR and TLC analysis.

Mono-pyrazoline derivative (13) and dipyrazoline derivatives (14a and b). A 100 ml soln of $CHCl_3$ containing 150 mg isabelin was mixed with 60 ml freshly distilled ethereal diazomethane prepared from N-methyl-N-nitroso-*p*-toluenesulfonamide (1·2 g) by the standard procedure. The reaction mixture was kept in the dark at room temp for 3 hr. The needles which precipitated (100 mg) were filtered off and designated (A); the filtrate was concentrated *in vacuo* to 25 ml. On standing overnight, the concentrate yielded a second crop of crystals (75 mg) which were designated (B).

The mother liquor from the second crop of crystals was evaporated to dryness, yielding a crystalline mass. Trituration of the residue with ether (5 ml) gave 57 mg of crystals which were labeled (C).

The (A) crystals represented a single substance by TLC analysis (R_f 0·12, silica gel G, CH_2Cl_2-MeOH 60:1); recrystallization from CH_2Cl_2 (5 ml) by slow evaporation of the solvent afforded small prisms containing one mole of CH_2Cl_2 as a crystalline solvate (by NMR). The CH_2Cl_2 could be removed by prolonged evacuation at room temp. Pure **14a** had the following properties: m.p. 169° (dec); IR bands (Nujol): 1750 (carbonyl), 1545 (weak), 1205, 995 and 905 cm^{-1}. [Found: C, 59·18; H, 5·67; O, 18·71; N, 16·45. $C_{17}H_{20}O_4N_4$ requires: C, 59·30; H, 5·82; O, 18·60; H, 16·35%].

The (B) crystals, which consisted of one major component by TLC analysis (with same system used for **14a**), yielded by preparative TLC separation 40 mg of a second dipyrazoline derivative (**14b**). Recrystallization of the crude material first from 4 ml of $CHCl_3$-MeOH, 10:1, and then from MeCN gave **14b**: m.p. 162° (dec), IR bands (Nujol): 1760 (carbonyls), 1600 (weak), 100 and 902 cm^{-1}. [Found: C, 59·22; H, 5·61; C, 18·76; N, 16·41. $C_{17}H_{20}O_4N_4$ requires: C, 59·30; H, 5·82; O, 18·60; N, 16·35%].

TLC analysis of the (C) crystals showed a mixture of **14b** (R_f 0·24) and a new substance (R_f 0·51). Preparative TLC separation gave 15 mg of the new compound; recrystallization from 0·3 ml of CCl_4-MeOH (20:1) afforded colorless needles containing CCl_4 as a crystalline solvate. Recrystallization from CH_2Cl_2 afforded pure crystals of **13**, m.p. 133° (dec); IR bands (Nujol): 1780 and 1740 (carbonyls), 1650 (weak), 1560, 1175, 994, 875 and 782 cm^{-1}. [Found: C, 63·73; H, 5·79; O, 21·25; N, 9·11. $C_{16}H_{18}O_4N_2$ requires: C, 63·50; H, 5·96; O, 21·20; H, 9·27%].

A mono-pyrazoline derivative (15) from dihydroisabelin (2). Dihydroisabelin (150 mg) in $CHCl_3$ (10 ml) was mixed with excess ethereal diazomethane. Although after 2 hr colorless needles precipitated, the mixture was allowed to stand overnight at room temp before the crystals were filtered off and washed with ether (10 ml); the long needles (141 mg), which represented a single substance by TLC. (R_f 0·49; silica gel G; CH_2Cl_2-MeOH, 100:3), contained one-half mole equivalent of ether (by NMR).

Recrystallization of the material from MeCN afforded pure crystals of **15**, m.p. 185–186° (dec); IR bands (Nujol): 1770 (carbonyls), 1610, 1207, 990 and 900 cm^{-1}. [Found: C, 63·62; H, 6·60; O, 21·26; N, 9·28. $C_{16}H_{20}O_4N_2$ requires: C, 63·20; H, 6·58; O, 21·20; N, 9·22%].

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