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OXIDIZED ARISTOLANE SESQUITERPENES FROM *ARISTOLOCHIA DEBILIS*

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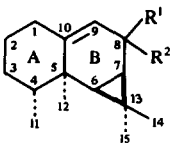
Key Word Index—*Aristolochia debilis*; Aristolochiaceae; structure elucidation; sesquiterpenes; aristolanes; 1(10)-aristolenal-(15); 9α-hydroperoxy-1(10) aristolenone-(2); 1α-hydroxy-9-aristolenone-(8).

Abstract—The underground parts of *Aristolochia debilis* have afforded three new aristolane type sesquiterpenes: 1(10)-aristolenal-(15), 1α-hydroperoxy-1(10)-aristolenone-(2) and 9α-hydroxy-9-aristolenone-(8).

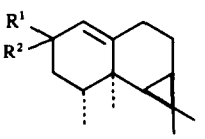
INTRODUCTION

The aristolane sesquiterpenes **1**–**5** [1–3] and oxoishwarane **7** [3] have been isolated from the underground parts of *Aristolochia debilis* Sieb. et. Zucc., a plant which is used in Chinese traditional medicine [4]. We now report the isolation of three new oxidized aristolanes (**6**, **9**, **10**), besides the known compound **8** [6], from a mixture of carbonyl compounds which was isolated from the petrol–diethyl ether extract of the dried underground parts with Girard P-reagent [5].



1 $R^1=R^2=H$

2 $R^1,R^2=O$



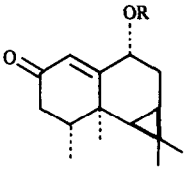
3 $R^1=R^2=H$

4 $R^1,R^2=O$

RESULTS AND DISCUSSION

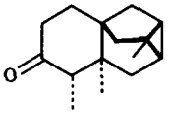
Compound **9**, $C_{15}H_{22}O$, has five double bond equivalents. The presence in its IR spectrum of carbonyl stretching at 1675 cm^{-1} indicates an α,β-unsaturated carbonyl group. However, in its UV spectrum the $n \rightarrow \pi^*$ transition is missing. Since, in the ^1H NMR spectrum (Table 1), the singlet of an aldehyde proton is located at δ9.57, the aldehyde group is obviously attached to a quaternary carbon. The IR shift of the aldehyde group to lower frequencies can be explained by the 'conjugation effect' of the cyclopropyl ring system as indicated by ^1J (^{13}C–^1H) coupling constants of 158 and 164 Hz for the cyclopropyl protons at δ44.8 and 30.8 in the ^{13}C NMR spectrum [8]. Further substructures are a trisubstituted double bond as well as three methyl groups, two attached to a quaternary C-atom and one attached to a tertiary C-atom. Among the sesquiterpenes with cyclopropane structures, maaliane and aristolane represent possible carbon skeletons for **9** [9]. However, maaliane can be excluded, because no signal for a methyl group linked to an olefinic double bond is observed in the ^1H NMR spectrum. The location of the double bond in the 1(10)-position of the aristolane skeleton is evident from a deshielding of C-6 in the ^{13}C NMR by 14 ppm relative to C-7, as well as from retro-Diels–Alder fragmentation in the mass spectrum forming *m/z* 176 (base peak).

Assignments of the ^{13}C NMR signals were achieved by comparison with the spectra of other aristolanes (Table 2). Directly bonded ^{13}C and ^1H nuclei were located by



5 R = H

6 R = OH



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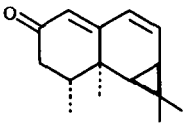

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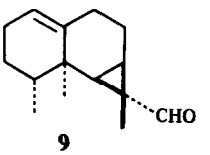

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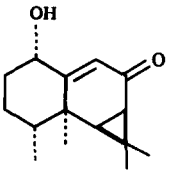

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Table 1. ^{1}H NMR spectral data of compounds **6**, **9** and **10** [$CDCl_3$ (30°), TMS as int. reference]

H	6	9	10
1	5.88 *s*	5.37 *quint*	4.39 *t*
2	—	1.49 *m*	1.60 *tt*
			2.01 $dddd_{eq}$
3	2.30 ddd_{eq}	1.99 *m*	1.7–1.9 *m**
	2.38 dd_{ax}		
4	2.31 *m**	1.37 *m*	
6	0.733 *d*	1.51 *d*	1.43 *d*
7	0.87 *td*	1.76 *ddd*	1.80 *dd*
8	1.78 ddd_{ax}	2.44 *m*	—
	2.39 ddd_{eq}	2.18 *m*	
9	4.32 *dd*	1.99 *m*	5.88 *d*
11	1.08 *d*	0.95 *d*	1.09 *d*
12	0.92 *s*	1.20 *s*	1.20 *s*
14	1.05 *s*	1.14 *s*	1.21 *s*
15	1.38 *s*	9.57 *s*	1.38 *s*
ROH	7.98 *s*	—	1.66 *s*

*Poorly resolved.

Table 2. ^{13}C NMR spectral data of compounds **2**, **4**, **6**, **8**, **9** and **10** ($CDCl_3$ (30°), values (ppm) relative to TMS)*

C	2	4	6	8	9	10
1	33.2 *t*	125.1 *d*	129.7 *d*	122.7 *d*	122.1 *d*	73.1 *d*
2	26.2 *t*	198.2 *s*	199.5 *s*	199.5 *s*	27.0 *t*	32.7 *t*
3	30.6 *t*	42.5 *t*	42.7 *t*	43.2 *t*	25.5 *t*	25.0 *t*
4	38.7 *d*	36.5 *d*	37.2 *d*	38.0 *d*	40.4 *d*	38.9 *d*
5	39.6 *s*	38.6 *s*	37.5 *s*	36.2 *s*	36.7 *s*	39.1 *s*
6	39.2 *d*	33.4 *d*	32.0 *d*	34.0 *d*	44.8 *d*	40.4 *d*
7	35.6 *d*	19.4 *d*	16.7 *d*	26.1 *d*	30.8 *d*	36.5 *d*
8	196.3 *s*	20.2 *t*	24.0 *t*	137.1 *d*	21.0 *t*	197.2 *s*
9	124.4 *d*	30.6 *t*	85.3 *d*	125.1 *d*	29.0 *t*	127.3 *d*
10	167.6 *s*	174.0 *s*	165.6 *s*	162.9 *s*	142.3 *s*	162.8 *s*
11	16.3 *q*	17.2 *q*	17.2 *q*	15.2 *q*	15.9 *q*	16.2 *q*
12	29.8 *q*	29.2 *q*	29.0 *q*	28.9 *q*	22.5 *q*	29.9 *q*
13	24.3 *s*	19.1 *s*	18.9 *s*	27.9 *s*	35.2 *s*	25.0 *s*
14	16.5 *q*	15.4 *q*	15.0 *q*	14.7 *q*	19.7 *q*	16.2 *q*
15	22.6 *q*	21.7 *q*	22.4 *q*	22.1 *q*	204.8 *d*	24.7 *q*

*Signals due to one-bond CH coupling as determined by single-frequency off-resonance or gated proton-decoupling.

selective proton decoupling. The deshielding of C-13 in the ^{13}C NMR spectrum of **9** by about 10 ppm when compared with **2** and **5**, supports the bonding of the aldehyde group to this carbon. In a gated decoupling experiment, the same signal showed a splitting of 20 Hz due to a two-bond ^{1}H–^{13}C coupling with the adjacent aldehyde proton. Further support for the position of the aldehyde group are downfield shifts of the cyclopropane protons (H-6, H-7) in the ^{1}H NMR spectrum to 1.51 ppm (*d*, $J_{6,7}$ = 9 Hz), and 1.76 ppm (*ddd*, $J_{6,7}$ = 9 Hz, $J_{7,8}$ = 10.5, 3 Hz), respectively. These shifts arise both from the double bond and the anisotropy of the carbonyl group. The *exo* position of the aldehyde group, corresponding to a *cis* configuration of the aldehyde and cyclopropane protons, is deduced from the aldehydes ^{1}H NMR signal forming a sharp singlet. A *transoidal* arrangement would cause this signal to be broadened or even split by *W*-coupling (^5J: ^{1}H–^{1}H). As the aldehyde group is in a position unusual for sesquiterpenes, **9** is formed biogenetically by oxidation of the *exo* methyl group (C-15).

The crystalline substance **6** ($C_{15}H_{22}O_3$) is an α,β-unsaturated carbonyl compound with a trisubstituted double bond, in accordance with the UV data: λ_{max} 231 nm ($\log\varepsilon$ 4.09) and the presence in the IR spectrum of an absorption band at 1650 cm^{-1}. The spectroscopic data, in general, are similar to those of debilone (**5**), into which **6** is readily converted upon standing. A positive reaction with potassium iodide/starch reagent indicates a hydroperoxy group. The hydroperoxy proton, which gives rise to a sharp singlet in the ^{1}H NMR spectrum (Table 1), can be exchanged by deuterium. A *W*-coupling, as observed in **5**, is not possible. C-9, to which the hydroperoxy group is bonded, appears at δ85.3 in the ^{13}C NMR spectrum (Table 2) [10]. Since the spectroscopic data did not lead to an unambiguous structure, the investigation was completed by X-ray structural analysis (Fig. 1), the results of which confirmed the position of the hydroperoxy group. A survey of the geometrical results shows all bond distances and angles to be normal, except for C-1/C-2 (1.454 Å) being somewhat shortened in the presence of the two adjacent double bonds C-1/C-10 (1.327 Å) and C-2/O-1 (1.222 Å). The atoms C-6, C-7, C-13 form an almost perfect equilateral triangle with bond angles very close to 60°.

Compound **6** is also obtained upon treatment of **4** with oxygen. Oxidation opposite to the cyclopropane ring is obviously preferred. The spectral data of compound **10** ($C_{15}H_{22}O_2$) correspond to those of aristolane (**2**) and debilone (**5**). In contrast to **2**, hydroxyl absorptions at

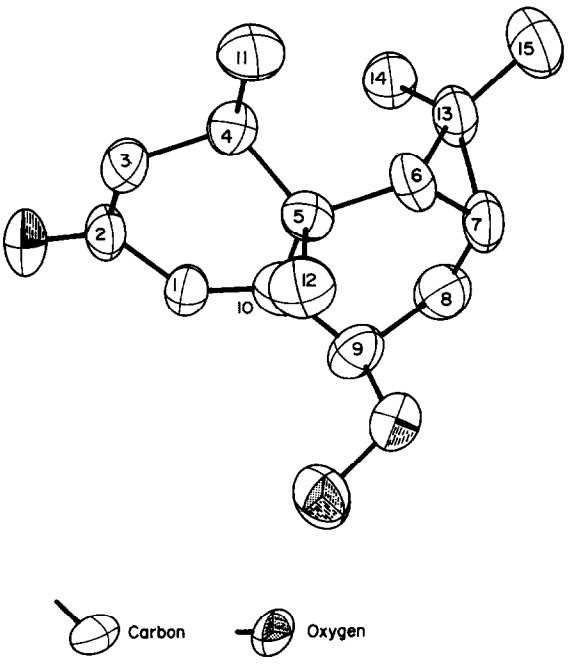

Fig. 1. ORTEP plot of compound **6**.

3430 and 3390 cm^{-1} are found in the IR spectrum. The ^1H NMR spectrum (Table 1) shows an ABX-system of H-6, H-7 and H-9, similar to that in **2**, H-6 and H-7 being cyclopropyl protons. The α,β-unsaturated carbonyl group has to be arranged in the same way as in **2**. An exchangeable proton at δ1.66 and a triplet at 4.39 (carbinol proton) locate a hydroxyl function in an allylic position.

In addition, signals belonging to three tertiary and one quaternary methyl groups are observed in the ^{13}C NMR spectrum (Table 2). Obviously **10** possesses the same aristolane structure as **2**, but additionally bears a hydroxyl group at C-1. The *axial* position of this hydroxyl group can be derived from the coupling constants of the carbinol proton with the methylene protons of C-2. In the ^{13}C NMR spectrum, the *endo* methyl group C-14 as well as C-4 are not influenced by the hydroxyl group; obviously this group is in the α-position of the chair conformation of ring A. The other ^1H NMR data (Table 1) are in accordance with this conformation.

EXPERIMENTAL

Mps: uncorr; TLC: silica gel; petrol (bp upto 40°).

Isolation of **6**, **9** *and* **10**. Dried, powdered underground parts (10 kg) of *A. debilis* were macerated (× 7) with 10 l. of petrol–Et_2O (1:1) to give 660 g of a yellowish-gold oil. After separation of an acidic fraction using 5% Na_2CO_3 soln, an 88% neutral fraction was obtained as a light yellow oil. Treatment of 240 g of the neutral fraction with Girard P-reagent [5] gave 198 g main fraction and 18 g carbonyl fraction. CC of 5 g of the carbonyl fraction (CH_2Cl_2–MeCOEt, 23:2) gave 0.75 g fraction C_a (R_f 0.59–0.78), 2.6 g fraction C_b (R_f 0.55–0.71) and 0.2 g fraction C_c (R_f 0.36–0.40). CC of fraction C_a (petrol–EtOAc–MeCOEt, 23:1:1) yielded 85 mg **9** (R_f 0.44) and 185 mg **7** (R_f 0.38). Prep. TLC of fraction C_b (petrol–EtOAc–MeCOEt, 86:7:7) gave 410 mg **4** (R_f 0.43), 60 mg **8** (R_f 0.39) and 1250 mg **2** (R_f 0.37). CC of fraction C_c (2-chloropropane–Me_2CO, 9:1) gave 20 mg **6** (R_f 0.36) and 55 mg **5** (R_f 0.27). CC of 20 g of the main fraction (CH_2Cl_2) gave fractions with R_f 0.27–0.73. Subsequent elution with EtOAc afforded 3.6 g of a fraction from 1.5 g of which 75 mg **10** were obtained by CC, using 2-chloropropane–Me_2CO (4:1).

*9α-Hydroperoxy-*1(10)*-aristolenone-*(2)([1*a*R,3R,7R,7*a*R,7*b*S]-1,1*a*,2,3,6,7,7*a*,7*b*-*Octahydro-3-hydroperoxy-1,1,7,7a-tetramethyl-5H-cyclopropa*(a)*-naphthalene-5-one*) (**6**). White prisms from EtO_2, mp 140°. UV λ_{max}^{MeOH} nm (log ε): 231 (4.09), 320 (2.85); IR ν_{max}^{KBr} cm^{-1}: 3240 (OOH); 3030 (cyclopropane), 3000 (C=CH), 1650 (C=O), 1620 (C=C); ^1H NMR (500 MHz): Table 1; ^{13}C NMR (250 MHz): Table 2; EIMS (Kratos MS 30, 150°) 70 eV *m/z* (rel. int.): 250 $[M]^+$ (3), 232 (17), 217 (23), 216 (18), 204 (10), 201 (14), 190 (68), 135 (53), 93 (50), 91 (58), 68 (76), 41 (100).

1(10)*-Aristolenal-*(15) ([1S,1*a*R,7R,7*a*R,7*b*R]-1*a*,2,3,5,6,7,7*a*,7*b*-*Octahydro-1,7,7a-trimethyl-1H-cyclopropa*(a)*-naphthalene-1-carb-aldehyde*) (**9**). White needles from Et_2O, mp 65°. Semicarbazone: white needles, mp 206°. UV $\lambda_{max}^{Et_2O}$ nm (log ε): 275 (2.18); IR ν_{max}^{KBr} cm^{-1}: 3020; 2780 and 2765 (HC=O), 1675 (C=O), 840 (C=CH); ^1H NMR (400 MHz): Table 1; ^{13}C NMR (400 MHz): Table 2; EIMS (Kratos MS 50, 150°) 70 eV *m/z* (rel. int.): 218.167 $[M]^+$ (12) ($C_{15}H_{22}O$), 217 (2), 203 (40), 189 (3), 176 (100), 175 (10), 147 (46), 145 (41), 119 (59), 118 (68), 105 (63).

1α*-Hydroxy-9-aristolenone-*(8)([1*a*R,4S,7R,7*a*R,7*b*S]-1*a*,4,5,6,7,7*a*,7*b*-*Octahydro-4-hydroxy-1,1,7,7a-tetramethyl-2H-cyclopropa*(a)*-naphthalene-2-one*) (**10**). White needles from Et_2O-pentane, mp 134°, UV $\lambda_{max}^{Et_2O}$ nm (log ε): 228 (4.05); IR ν_{max}^{KBr} cm^{-1}: 3430 and 3390 (OH), 3010 and 2995 (C=CH), 1660 (C=C), 1640 (C=O); ^1H NMR (250 MHz): Table 1; ^{13}C NMR (250 MHz): Table 2; EIMS (Kratos MS 50, 150°) 40 eV *m/z* (rel. int.): 234.162 $[M]^+$ (39) ($C_{15}H_{22}O_2$), 219 (19), 216 (71), 201 (95), 149 (44), 145 (28), 131 (30), 121 (34), 119 (38), 105 (69), 40 (100).

X-Ray crystallography. The crystallographic data for **6** were collected in an SYNTEX $P2_1$ diffractometer. The space group was found to be $P2_12_12_1$ with cell constants a = 9.582(6) Å, b = 11.785(8) Å, c = 12.297(9) Å, and four molecules in the unit cell. The intensities of 1431 reflections were measured using graphite monochromated MoK_α-radiation. 826 unique reflections with $I > 2.5\,\sigma(I)$ were used in the structure determination. The structure was solved by use of MULTAN 80, and refined by least squares methods to R = 0.062, R_w = 0.023 ($w = 1/\sigma^2(F)$). [In the final stage of refinement the hydrogen atom positions were calculated, the hydrogens were included in the model and refined for their positions only with fixed isotropic temperature factors B(H) = 1.2· B(C).] Figure 1 is an ORTEP drawing of the molecule. Listings of the atomic coordinates, bond distances and angles, and observed relative structure amplitudes are deposited with the Cambridge University Crystallographic Centre.

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REFERENCES

1. Křepinský, J., Jommi, G., Samek, Z. and Šorm, F. (1970) *Collect. Czech. Chem. Commun.* **35**, 745.
2. Büchi, G., Greuter, F. and Tokoroyama, T. (1962) *Tetrahedron Letters* 827.
3. Nishida R. and Kumazawa Z. (1973) *Agric. Biol. Chem.* **37**, 341.
4. Perry L. M. (1980) in *Medicinal Plants of East and Southeast Asia*. MIT Press, Cambridge, MA.
5. Girard, A. and Sandulesco, G. (1936) *Helv. Chim. Acta* **19**, 1095.
6. Rücker, G. (1968) *Liebigs Ann. Chem.* **717**, 221.
7. Nakanishi, K. (1962) in *Infrared Absorption Spectroscopy*. Holden Day, San Francisco.
8. Baum, M. W., Guenzi, A., Johnson, C. A. and Mislow, K. (1982) *Tetrahedron Letters* 31.
9. Rücker G., (1979) in *Vorkommen und Analytik ätherischer Öle*. (Kubeczka, K., ed.) p. 144. Georg Thieme, Stuttgart.
10. Bremser W., Franke B. and Wagner H. (1982) in *Chemical Shift Ranges in Carbon-13-NMR-Spectroscopy*. Verlag Chemie, Weinheim.