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BUDDLEDIN A, B AND C, PISCICIDAL SESQUITERPENES FROM *BUDDLEJA DAVIDII* FRANCH.

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Some plants of *Buddleja* species have been known to be poisonous to fishes. We have isolated from the root bark of *Buddleja davidii* Franch. (Buddlejaceae), three new piscicidal sesquiterpenes, buddledin A (1), B (2) and C (3).

Chromatography over silica gel followed by preparative TLC guided by the killie-fish test[1], gave buddledin A, B and C in *ca.* 0.5, 0.003 and 0.03% yields (from the fresh root bark), whose spectral data are listed in Table 1.

Buddledin A, $C_{17}H_{24}O_3$ (M^+ 276), m.p. 94-95°, $[\alpha]_D$ -245° (c, 0.74, $CHCl_3$), shows in the spectra the presence of an α,β-unsaturated ketone substituted by CH_3 at α- and by CH_2 at β-carbon (UV 238 nm, IR, 1682, 1642 cm^{-1}, PMR, β-H at δ 6.45 (m) coupled with α-CH_3 at δ 1.64 (d, J=2 Hz). An acetyl group (IR, 1742, 1236 cm^{-1}, PMR, δ 2.14) on a secondary carbon whose proton (H_A, δ 5.67, d, J=11 Hz, shifted to δ 4.69 on deacetylation of 1), is coupled with another methine proton (H_B, t, J=11 Hz) which is resolved by addition of $Eu(fod)_3$, is also exhibited. A two-proton singlet at δ 4.93 of exomethylene group (IR, 890 cm^{-1}), replaced by a secondary methyl signal at δ 0.83 (d, J=7 Hz) upon hydrogenation of 1 to tetrahydrobuddledin A (4), $C_{17}H_{28}O_3$ (M^+ 280), m.p. 88-89°, is shown by addition of the shift reagent to be coupled with allylic H_C (br.q) which is coupled with H_B. Two tertiary methyl groups (PMR, δ 1.11 and 1.10, CMR[2], two of three signals at δ 13.7, 20.6 and 21.6) on a quaternary carbon (CMR, δ 34.0), and two additional methylene carbons (CMR, two of three peaks at δ 30.0, 40.0 and 41.3) are also exhibited. The *gem*-dimethyl-carrying carbon and one of the methylene groups are presumed to be located adjacent to H_B- and H_C-carrying carbon, respectively, on the basis of the splitting patterns of these protons (H_B, t, and H_C, br.q). The AcO-carrying carbon is shown to be α to the ketone group by the $LiAlH_4$ reduction of 1 to epimeric alcohols, 5, $C_{15}H_{24}O_2$ (M^+ 236), m.p. 123-125°, and 6, m.p. 70-72°, which show coupling of newly formed H_D with H_A [5, H_A δ 3.75 (dd, J=2, 10 Hz), H_D δ 4.17 (d, J=2 Hz); 6, H_A δ 3.74 (t, J=9 Hz), H_D δ 3.50 (d, J=9 Hz)]. The couplings in these PMR data were confirmed by PMDR. These spectral data indicate partial structure 1a, and coupled with the biogenetic considerations, lead to structure 1 for buddledin A.

The structure and relative stereochemistry including the geometry of the endocyclic double bond as depicted in 1 were unequivocally established by X-ray diffraction analysis of the bromohydrin 7, m.p. 179-180°, which was derived from 1 by the treatment with N-bromoacetamide in aqueous acetone. Clear crystals of 7 belong to the orthorhombic space group $P2_12_12_1$ with unit cell

axes a=19.747, b=8.693, c=10.194 Å, and four molecules of $C_{17}H_{25}O_4Br$ per unit cell. Of 1879 reflections, collected with a Syntex $P\bar{1}$ automated diffractometer, 1133 were above the background (2 σ). The structure was solved by the heavy atom method and refined by the full-matrix least-squares method to a conventional R-value of 0.078 without hydrogen atoms.

The absolute configuration as given in 1 was determined by the application of dibenzoate chirality rule[3] and benzoate rule[4] as follows. CD spectra of dibenzoate 8, $C_{29}H_{32}O_4$ (M^+ 444), m.p. 129-130°, PMR, δ 5.36 (dd, J=3, 10 Hz, C_2-H), 5.67 (d, J=3 Hz, C_3-H), and 9, $C_{29}H_{32}O_4$ (M^+ 444), m.p. 122-123°, PMR, δ 5.64 (t, J=10 Hz, C_2-H), 5.31 (d, J=10 Hz, C_3-H), obtained from two epimeric diols 5 and 6, show positive ($\Delta\varepsilon_{236}$ +20.5, $\Delta\varepsilon_{220}$ -3.5) and negative ($\Delta\varepsilon_{237}$ -15.25, $\Delta\varepsilon_{223}$ +19.0) chiralities, which indicate β orientation of C_2-OH. Application of the benzoate rule for monoalcohol 13, $C_{15}H_{28}O$ (M^+ 224), $[M]_D$ +13.2°, derived from 4 by four-steps sequence (4 → 10 → 11 → 12 → 13), and its benzoate 14, $C_{22}H_{32}O_2$ (M^+ 328), $[M]_D$ +207.8°, agrees with the result from the dibenzoate chirality rule.

Buddledin B, $C_{15}H_{22}O_2$ (M^+ 234), m.p. 139-141°, $[\alpha]_D$ -314° (c, 0.54, $CHCl_3$), was proved to be deacetylbuddledin A (2) obtainable by deacetylation of 1. Ethyl ether 15, $C_{17}H_{28}O_3$ (M^+ 280), m.p. 95-96°, along with 2, was produced upon the ethanolysis. Acetylation of 2 regenerated buddledin A.

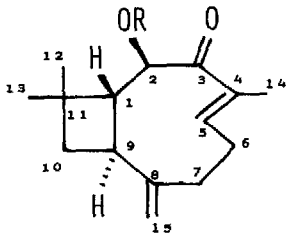
The spectroscopic data of buddledin C, $C_{15}H_{22}O$ (M^+ 218), m.p. 129-130°, $[\alpha]_D$ -316° (c, 0.54, $CHCl_3$) suggest it to be deacetoxybuddledin A (3) and this has been confirmed as follows. With Zn-AcOH, buddledin A was converted to deacetoxydihydrobuddledin A (16), $C_{15}H_{24}O$ (M^+ 220), $[\alpha]_D$ +42.6° (c, 0.27, dioxane), and dihydrobuddledin A (17), $C_{17}H_{26}O_3$ (M^+ 278). The former was

Table 1. Spectral Data of 1, 2, 3 and Their Derivatives

Compound	ν_{max}^{KBr}, cm^{-1}	λ_{max}^{MeOH}, nm (ε)	PMR (90 MHz, $CDCl_3$, δ in ppm, J in Hz)				
			H-2	H-5	H-12, 13	H-14	H-15
1	1742, 1682, 1642	238	5.67	6.45	1.11 (s)	1.64	4.93 (s)
	1236, 890	(8,500)	(d, 11)	(m)	1.10 (s)	(d, 2)	
2	3440, 1655, 1635	237	4.69	6.42	1.29 (s)	1.69	4.90 (s)
	1615, 903	(9,400)	(q, 5, 11)[a]	(m)	1.09 (s)	(d, 2)	
3	1662, 1637, 887	236	3.0-1.5[b]	6.33	1.00 (s)	1.63	4.92 (s)
		(11,000)		(m)		(d, 2)	4.95 (s)
18	1667, 880	235	2.7-1.5[b]	5.53	1.02 (s)	1.77	4.88 (br.s)
		(4,700)		(m)	1.01 (s)	(q, 1~2)	4.82 (br.s)
19	1738, 1707, 1668	237	5.27	5.60	1.13 (s)	2.01	4.85 (s)
	1633, 1236, 895	(3,500)	(d, 11)	(m)	1.09 (s)	(d, 1)	

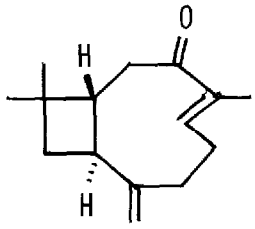
(a) Transformed to a doublet (J=11 Hz) on addition of D_2O.

(b) Overlapped by other protons.



1 R=Ac

2 R=H



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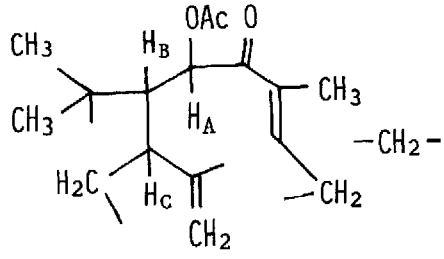
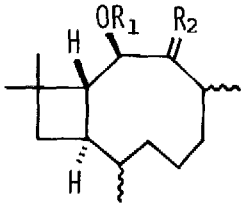


1a



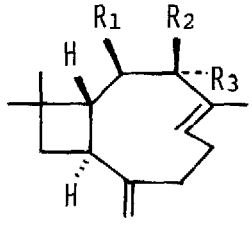
4 R_1=Ac, R_2=O

10 R_1=Ac, R_2= 

11 R_1=Ac, R_2= 

13 R_1=H, R_2=H_2

14 R_1=Bz, R_2=H_2

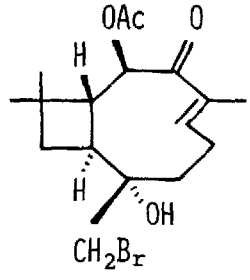


5 R_1=R_2=OH, R_3=H_D

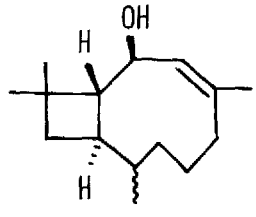
6 R_1=R_3=OH, R_2=H_D

8 R_1=R_2=OBz, R_3=H

9 R_1=R_3=OBz, R_2=H



7



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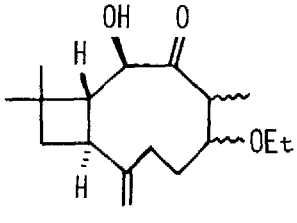

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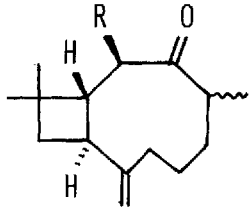
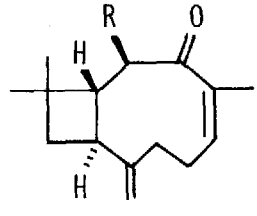


16 R=H

17 R=OAc



18 R=H

19 R=OAc

identified with the product (dihydrobuddledin C) obtained by the same treatment of buddledin C. On Zn-AcOH reduction in diluted solution in a shorter time, 3 afforded, in addition to 16, an isomer 18, $C_{15}H_{22}O$ (M^+ 218), $[\alpha]_D$ -199° (c, 0.7, $CHCl_3$), which was converted to 16 on prolonged reaction. This isomer was also obtained together with 16 and 17 by an analogous reaction of 1. Buddledin A was slowly isomerized in MeOH or Me_2CO solution at room temperature to give a *cis* isomer 19, $C_{17}H_{24}O_3$ (M^+ 276). This isomerization occurred quickly by the UV irradiation of 1 in *n*-hexane, as found with caryophyllene[5]. The weak UV absorption of α,β-unsaturated ketone in 19 and the upfield shift of C_5-olefinic proton in PMR spectrum would be due to torsion of the conjugated system in the *cis* isomer. The isomerization of 3 → 18 has been indicated to be from *trans* to *cis* by the analogous change in the spectra (Table 1).

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2. 22.6 MHz, $CDCl_3$, δ in ppm rel. to TMS.
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