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Studies on the Constituents of *Buddleja* Species. I. Structures of Buddledin A and B, Two New Toxic Sesquiterpenes from *Buddleja davidii* Franch.[1]

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Two new piscicidal sesquiterpenes, buddledin A (**1**) and B (**2**), have been isolated from the root bark of *Buddleja davidii* Franch. These structures have been deduced on the basis of spectroscopic and chemical evidences, and confirmed by X-ray analysis of the bromohydrin (**8**). The absolute configuration was determined by the applications of benzoate rule and dibenzoate chirality rule. Buddledin A and B are the first examples of toxic compounds possessing the caryophyllene skeleton.

Keywords——*Buddleja davidii* Franch.; Buddlejaceae; piscicidal sesquiterpene; caryophyllene derivative; buddledin A; buddledin B; UV, IR and NMR spectra; X-ray analysis; absolute configuration

Some plants of *Buddleja* species (Buddlejaceae) have been known to be toxic to fishes, but no toxic principle of this genus has been isolated. We have isolated two crystalline constituents, named buddledin A (**1**) and B (**2**), which are toxic to killie-fish, from methanolic extract of root bark of *Buddleja davidii* Franch.

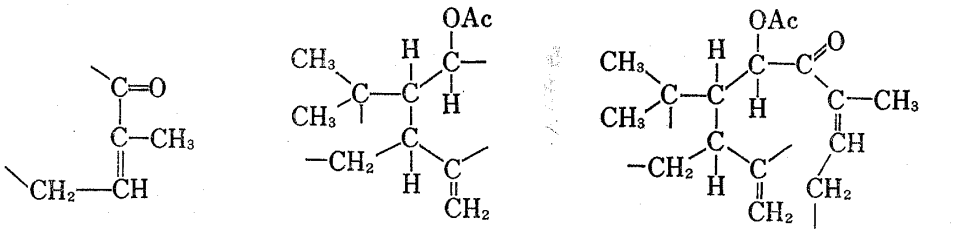
The fresh root bark was extracted with methanol and the concentrated extract was then partitioned between ether and water. Column chromatography of the ethereal extract, followed by preparative thin-layer chromatography (prep. TLC) led to the isolation of the main toxic principle, buddledin A, mp 94—95°, $C_{17}H_{24}O_3$, and another toxic principle, buddledin B, mp 139—141°, $C_{15}H_{22}O_2$. Buddledin A was shown to be an acetate of buddledin B as the former was produced upon acetylation of the latter.

Buddledin A shows infrared (IR) and ultraviolet (UV) absorptions characteristic of conjugated ketone [UV λ_{max}^{MeOH}, 238 nm (ε 8500), IR ν_{max}^{KBr}, 1682, 1642 cm^{-1}]. In the proton nuclear magnetic resonance (PMR) spectrum, two tertiary methyl (δ 1.11, 1.10) and a vinyl methyl (δ 1.64, br.s) which is coupled with an olefinic proton at δ 6.45 (m) assignable to the β-proton of α,β-unsaturated ketone system, are exhibited. The irradiation at δ 1.64 collapses the signal of olefinic proton into a double doublet (J=10, 5 Hz), indicating the partial structure (i). The PMR spectrum also exhibits an acetate methyl signal at δ 2.14 and a one-proton doublet (J=11 Hz) at δ 5.67, which is assignable to a proton (H-2) attached to the carbon bearing an acetoxyl group. This doublet was shifted upfield to δ 4.67 (d, J=11 Hz) upon alcoholysis of buddledin A to buddledin B. The presence of an exomethylene group (IR ν_{max}^{KBr}, 890 cm^{-1}) is showh by a two-proton singlet at δ 4.93, which was replaced by a secondary methyl signal at δ 0.83 (d, J=7 Hz) in the tetrahydrobuddledin A (**3**), mp 88—89°, $C_{17}H_{28}O_3$, obtained by the catalytic hydrogenation of buddledin A. The ^{13}C nuclear magnetic resonance (CMR) spectrum [22.6 MHz, $CDCl_3$, δ (ppm) relative to tetramethylsilane (TMS), multiplicities by off-resonance decoupling in parentheses] exhibits four methyl carbons at δ 13.7, 20.6, 21.6 and 30.7, three methylene carbons at δ 30.0, 40.0 and 41.3, three methine carbons at δ 45.0,

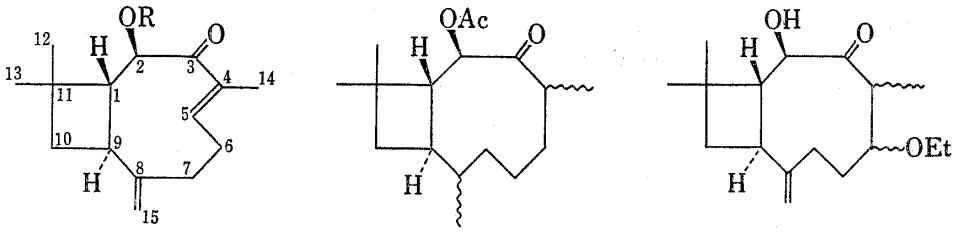
1) A short preliminary communication: T. Yoshida, J. Nobuhara, M. Uchida, and T. Okuda, *Tetrahedron Lett.*, **1976**, 3717.

2) Location: *1-1-1, Tsushima-naka, Okayama 700, Japan.*

56.2 and 79.4, one quaternary carbon at δ 34.0, four olefinic carbons at δ 111.7 (t), 134.6 (s), 142.4 (d) and 151.0 (s) and two carbonyl carbons at δ 169.5 and 197.3. The presence of one quaternary carbon in the molecule suggests that the two tertiary methyl groups should be forming a *gem*-dimethyl group. As there is no evidence of further unsaturation, buddledin A should be bicarbocyclic.

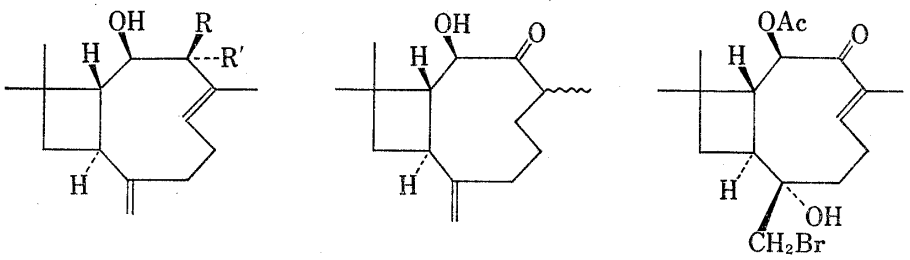


(i) (ii) (iii)



1 : R=Ac
2 : R=H

3 **4**



5 : R=OH, R'=H
6 : R=H, R'=OH

7 **8**

Chart 1

Ethanolysis of buddledin A afforded an ethyl ether (**4**), mp 97—98°, $C_{17}H_{28}O_3$, along with the alcohol (**2**). Examination of the PMR spectrum of **4** on gradual addition of Eu(fod)$_3$ shift reagent provided an evidence for partial structure (ii) in buddledin A. At a concentration of 0.2 molar equivalent of Eu(fod)$_3$ in $CDCl_3$, H-1 (δ 3.09, t, J=11 Hz) and H-9 (δ 3.66, br.q) were clearly distinguished from other protons. Irradiation of H-2 (δ 6.01, d, J=11 Hz) transformed H-1 into a doublet (J=11 Hz). Irradiation at δ 3.66 likewise produced a doublet (J=11 Hz) for H-1 and also collapsed the exomethylene signals at δ 4.82 (t, J=2 Hz) and 4.97 (br.s, $W_{h/2}$=5 Hz) to a pair of broad singlet ($W_{h/2}$=4 Hz). Partial structures (i) and (ii) were combined to form (iii) based on the following evidences. Buddledin A was reduced with $LiAlH_4$ to give two epimeric diols, (**5**), mp 123—125°, $C_{15}H_{24}O_2$ and (**6**), mp 70—72°, $C_{15}H_{24}O_2$, whose PMR spectra showed coupling of newly formed H-3 with H-2 [**5**, H-2, δ 3.75 (dd, J=3, 10 Hz), H-3, δ 4.15 (d, J=3 Hz); **6**, H-2, δ 3.74 (t, J=9 Hz), H-3, δ 3.50 (d, J=9 Hz)]. The couplings in these PMR data were confirmed by decoupling experiments. Deacetyldihydrobuddledin A (**7**), besides **5** and **6**, was produced upon this $LiAlH_4$ reduction. Based on these data and the biogenetic considerations, structure **1** is presumed for buddledin A.

Structure **1** including the relative stereochemistry and the geometry of the endocyclic double bond was established by X-ray diffraction analysis of the bromohydrin (**8**), mp 185—186°, $C_{17}H_{25}BrO_4$, which was obtained by the treatment of **1** with N-bromoacetamide in aqueous acetone. The crystal structure was solved by the heavy atom method and refined to a final R value of 0.078 for 1133 non-zero reflections measured on a Syntex $P\bar{1}$ four-circle diffractometer (Mo$K\alpha$ radiation). A computer generating drawing[3] of the final X-ray model and the bond lengths (in angstrom unit) and angles (in degrees) are presented in Fig. 1 and 2. The average standard deviations of these values are estimated to be 0.018 Å for C-C lengths and 1.1° for C-C-C angles.

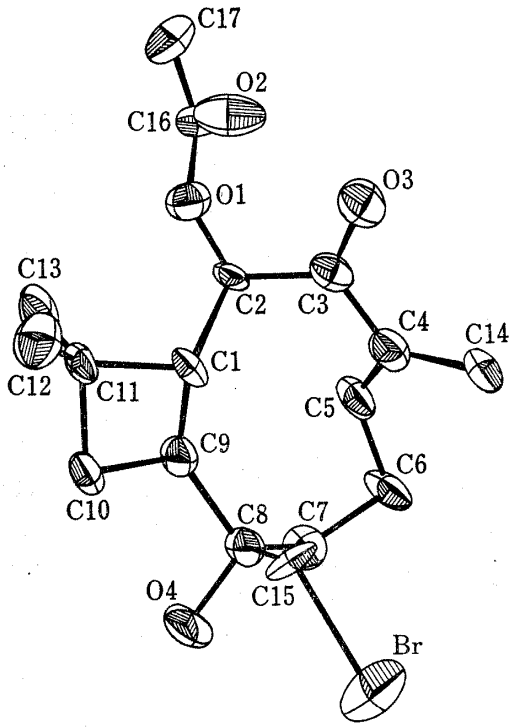


Fig. 1. An ORTEP Drawing of Bromohydrin (**8**)

The absolute configuration as given in **1** was determined in the following two ways. Firstly, the benzoate rule[4] was applied to the monoalcohol (**12**) and its benzoate (**13**). Monoalcohol (**12**), $C_{15}H_{28}O$, was prepared from the tetrahydrobuddledin A (**3**)[5] by the four-steps reaction sequence as shown in Chart 2. The molecular rotation difference, $[M]_{D(13)}-[M]_{D(12)}=+194.6°$, suggests that the hydroxyl group at C_2 in **12** is β-oriented.

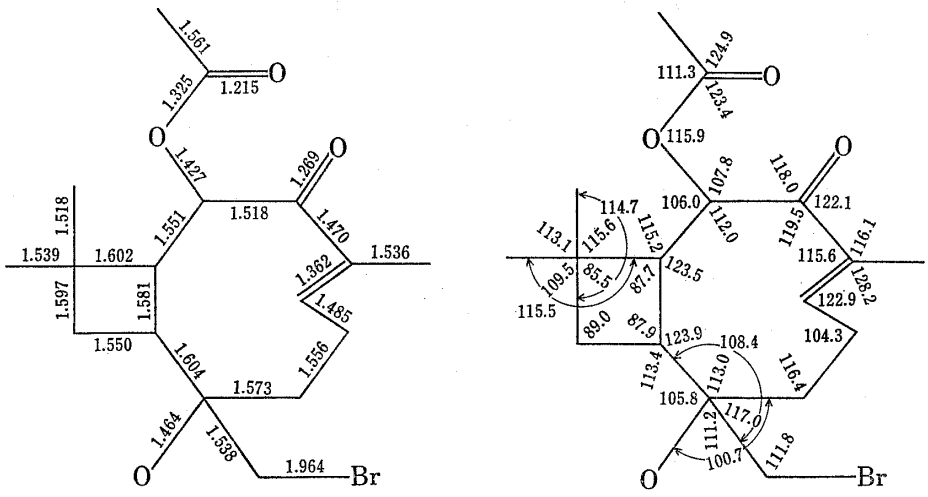
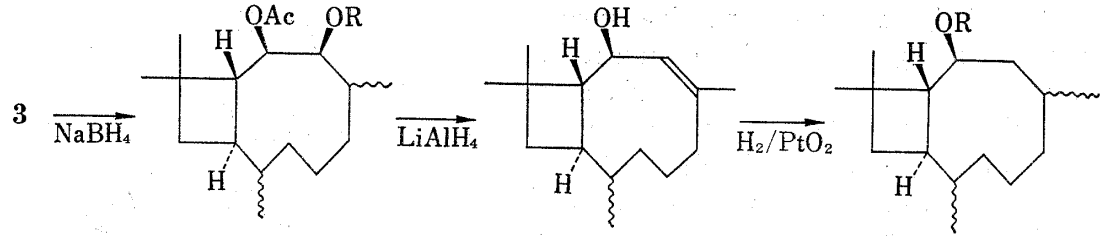


Fig. 2. Intramolecular Bonding Parameters for Bromohydrin (**8**)

Secondly, the application of the dibenzoate chirality rule[6] led to the same conclusion. Successful application of the dibenzoate chirality rule to the dibenzoxycycloheptane and dibenzoxycyclooctane has been reported.[7] Although the 1,2-diol in **5** and **6** is on the nine-

3) C. Johnson, "ORTEP, A Fortran Thermal-Ellipsoid Plot Programs," U.S. Atomic Energy Commission Report ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1965.
4) J.H. Brewster, *Tetrahedron*, **13**, 106 (1961).
5) Although the configurations at C_4 and C_8 of **3** and **12** are unknown, the PMR spectra of these compounds show that they are not accompanied by the diastereoisomers.
6) N. Harada and K. Nakanishi, *J. Amer. Chem. Soc.*, **91**, 3989 (1969).
7) Y. Yamamoto, M. Fushimi, J. Oda, and Y. Inouye, *Agr. Biol. Chem.*, **39**, 2223 (1975).



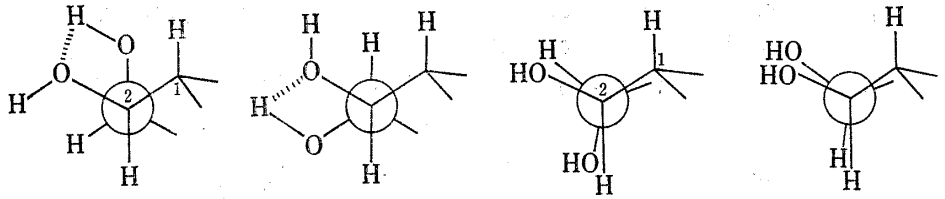
9: R=H
10: R=Ms

11

12: R=H
13: R=Bz

Chart 2

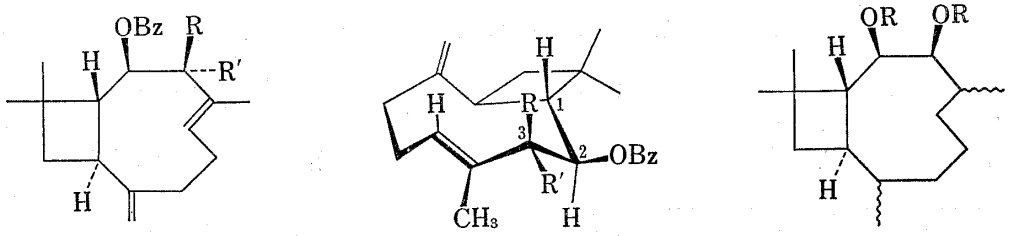
membered ring, the diol on this ring whose flexibility is partialy restricted by the fusion with the cyclobutane ring, is presumed to be *cis* in **5** and *trans* in **6**, based on the vicinal coupling constants ($J_{H1,H2}$=10 Hz, $J_{H2,H3}$=3 Hz in **5**, $J_{H1,H2}$=$J_{H2,H3}$=9 Hz in **6**) between protons at C_1—C_3, and also on the presence of a strong IR band due to the intramolecular hydrogen bonding in dilute carbon tetrachloride solution of each diol [**5**, 3630 (unbonded hydroxyl) and 3580 cm^{-1} (intramolecularly bonded hydroxyl), $\Delta\nu$ 50 cm^{-1}; **6**, 3635 and 3590 cm^{-1}, $\Delta\nu$ 45 cm^{-1}]. This hydrogen bonding appears to be inconsistent with the reversed *cis-trans* correlation as shown by **5a** and **6a**, as the hydrogen bonding is impossible for **5a** in which the conformational correlation of the diol is analogous to that in *trans*-cyclopentane-1,2-diol.[8]



5 (*cis*) **6** (*trans*) **5a** (*trans*) **6a** (*cis*)

Chart 3

The diols, **5**, and **6**, were benzoylated to give the dibenzoates **14**, mp 129—130°, $C_{29}H_{32}O_4$, PMR δ: 5.36 (dd, J=10, 3 Hz, H-2), 5.67 (d, J=3 Hz, H-3), and **15**, mp 122—123°, $C_{29}H_{32}O_4$, PMR δ: 5.31 (d, J=10 Hz, H-3), 5.64 (t, J=10 Hz, H-2). The analogy of the coupling constants ($J_{H1,H2}$ and $J_{H2,H3}$) in **14** and **15** to those of **5** and **6** indicates the conformations depicted by **14a** and **15a**, in which the orientation of the vinyl methyl group is arbitrary. The circular dichroism (CD) spectra of the dibenzoates (**14**) and (**15**) are shown in Fig. 3. The first Cotton effect is positive ($\Delta\varepsilon_{236}$ +20.5) in **14** and negative ($\Delta\varepsilon_{237}$ −15.25) in **15**, which



14: R=OBz, R'=H
15: R=H, R'=OBz

14a: R=OBz, R'=H
15a: R=H, R'=OBz

16: R=H
17: R=Bz

Chart 4

8) E.L. Eliel, "Stereochemistry of Carbon Compounds," McGraw-Hill Book Co., New York, 1962, p. 277.

indicates the chirality between the two benzoate groups is positive in **14** and negative in **15**. The *cis*-diol (**16**), mp 82—84°, $C_{15}H_{28}O_2$, obtained by $LiAlH_4$ reduction of **3**, was converted to the dibenzoate (**17**), $C_{29}H_{36}O_4$, whose CD spectrum also showed positive chirality ($\Delta\varepsilon_{237}$+5.1). These facts are in agreement with the absolute configurations as shown by **14**, **15** and 17.

Therefore, the absolute configurations of buddledin A and B have been established to be **1** and **2**.

The piscicidal activities of **1** and **2** were evaluated using killie-fish (*Oryzias laptipes*).[9] The median tolerance limit (TLm) after 24 hr was 0.6 ppm for **1**, and 1.2 ppm for **2**.

The isolation of buddledin A and B is the first example of the toxic principle in *Buddleja* species and they are also the first examples of toxic caryophyllene derivatives.

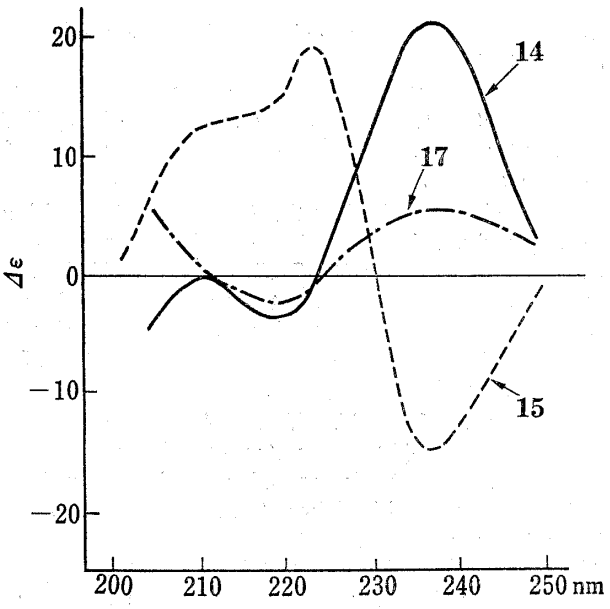


Fig. 3. CD Spectra of **14** (———), **15** (------) and **17** (-·-·-·-) in EtOH

Experimental

UV spectra were measured on a Shimadzu Multiconvertible Spectrophotometer Model Double-4OR in MeOH solutions, IR spectra on a JASCO Model IR-G Spectrophotometer. IR absorptions by the intramolecular hydrogen bonding were recorded using a quartz cell (l=1 cm) in solution of 0.005 and 0.0025 mol in CCl_4. PMR spectra were measured in $CDCl_3$ unless otherwise stated on a Hitachi R-22 (90 MHz) Spectrometer and chemical shifts (δ) are given in ppm relative to TMS as an internal standard. CMR spectra were recorded with NEVA's NV-21 (22.6 MHz) with ^2H internal lock. Mass Spectra (MS) were obtained with a Shimadzu-LKB 9000 GC-MS equipped with 2 m×3 mm i.d. glass column containing 2.5% OV-17 on 60—80 mesh Chromosorb W and direct inlet system (ion source temperature 270°, separator temperature 250°, electron energy 70 eV). Optical rotations were measured on a JASCO DIP-4 Digital Polarimeter in $CHCl_3$ solutions at room temperature. CD spectra were recorded on a JASCO J-20 Spectropolarimeter. Silicic acid (100 mesh, Mallinckrodt, U.S.A) was used for column chromatography and Kieselgel G and PF_{254} (E. Merck A. G., Germany) were used for TLC and prep. TLC. Materials were detected by UV light or spraying with 20% H_2SO_4 and charring. Solvent systems used for TLC: solvent I, benzene–AcOEt (9: 1); solvent II, $CHCl_3$–*n*-hexane (1: 1). The organic solutions were dried over $MgSO_4$ and evaporated in rotary evaporator under 50°.

Isolation of Buddledin A and B——The fresh root bark (1.45 kg) of *Buddleja davidii* Franch. collected in May at Okayama University campus was ground and soaked in MeOH (8 l) three times for several days each at room temperature. The combined MeOH extracts were concentrated to *ca.* 1 l, diluted with H_2O and were extracted with ether (750 ml×4). The ether layer was dried and evaporated to afford a dark brown residue (24 g), which was chromatographed over silica gel column (670 g) using benzene as eluant. The toxic activity was shown upon the killie-fish bioassay by the two fractions [*Rf* 0.45 and *Rf* 0.50 (TLC, solvent I)]. The fraction of *Rf* 0.45 was evaporated to give crude buddledin A. Recrystallization from MeOH–H_2O afforded buddledin A (4.33 g) as colorless plates, mp 94—95°. $[\alpha]_D$ −245° (*c*, 0.74). IR ν_{max}^{KBr} cm^{-1}: 1742, 1682, 1642, 1236, 890. UV λ_{max} nm (ε): 238 (8500). PMR δ: 1.10, 1.11 (each 3H, s), 1.64 (3H, br. s), 2.14 (3H, s, OAc), 4.93 (2H, s, H-15), 5.67 (1H, d, J=11 Hz, H-2), 6.45 (1H, m, H-5). MS *m/e*: 276 (M^+). *Anal.* Calcd. for $C_{17}H_{24}O_3$: C, 73.88; H, 8.75. Found: C, 73.97; H, 8.70.

The fraction of *Rf* 0.50 was revealed to be a mixture of at least two components (*Rf* 0.54 and 0.46) by TLC developed with solvent II. Purification by prep. TLC (solvent II) gave crude buddledin B (*Rf* 0.46), which was recrystallized from MeOH to yield colorless prisms, mp 139—141° (39 mg). $[\alpha]_D$ −314° (*c*, 0.54). IR ν_{max}^{KBr} cm^{-1}: 3440, 1655, 1635 (sh), 903. UV λ_{max} nm (ε): 237 (9400). PMR δ: 1.09, 1.28 (each 3H, s), 1.68 (3H, br. s, H-14), 4.67 [1H, dd, J=5, 11 Hz, converted into doublet (J=11 Hz) on addition of D_2O, H-2],

9) T. Okuda, T. Yoshida, S. Koike, and N. Toh, *Phytochemistry*, **14**, 509 (1975).

4.87 (2H, s, H-15), 6.39 (1H, m, H-5). MS *m/e*: 234 (M^+). *Anal.* Calcd. for $C_{15}H_{22}O_2$: C, 76.88; H, 9.46. Found: C, 76.92; H, 9.67.

After extraction with MeOH at room temperature, the residual root bark was further extracted with boiling MeOH (7 l×2), and further crop of buddledin A (3.28 g) was obtained from the ethereal extract (10.2 g) by the same procedure as described above. The total amount of buddledin A was 7.6 g (0.5% of the fresh root bark).

Acetylation of Buddledin B——A solution of buddledin B (40 mg) in a mixture of pyridine (1 ml) and Ac_2O (1 ml) was left standing overnight at room temperature and the reaction mixture was poured into ice-water and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with 1 N HCl, satd. aq. $NaHCO_3$ and satd. aq. NaCl, dried and evaporated to give a colorless syrup (36 mg). Purification by prep. TLC (solvent II) gave an acetate (20.6 mg), along with the starting material (4.2 mg). The acetate was recrystallized from MeOH–H_2O to give colorless needles, mp 94—96°, $[\alpha]_D$ −233° (*c*, 0.69), which was identified with buddledin A (mixed mp, IR, PMR, UV).

Hydrogenation of Buddledin A——Buddledin A (200 mg) in EtOH (30 ml) was hydrogenated over prereduced PtO_2 (40 mg) at atmospheric pressure for 3 hr. The catalyst was filtered off and the filtrate was evaporated. Crystallization of the residue from MeOH–H_2O provided tetrahydrobuddledin A (**3**) (100 mg), which was recrystallized from the same solvent to afford colorless needles, mp 88—89°. $[\alpha]_D$ −46.9° (*c*, 0.98). IR ν_{max}^{KBr} cm^{-1}: 1718, 1735 (shoulder), 1238, 965. PMR δ: 0.83 (3H, d, J=7 Hz, H-15), 1.10 (6H, s), 1.11 (3H, d, J=7 Hz, H-14), 2.04 (3H, s, OAc), 5.15 (1H, d, J=12 Hz, H-2). MS *m/e*: 280 (M^+). *Anal.* Calcd. for $C_{17}H_{28}O_3$: C, 72.82; H, 10.06. Found: C, 72.75; H, 10.32.

The mother liquor was purified by prep. TLC (solvent I) to give additional crop of **3** (58 mg) and hexahydrobuddledin A (**9**) (5 mg), mp 117—118° (MeOH–H_2O). $[\alpha]_D$ +36.1° (*c*, 0.46). IR ν_{max}^{KBr} cm^{-1}: 3450, 1710, 1258. PMR δ: 0.89, 0.97 (each 3H, d, J=8 Hz), 0.96, 1.02 (each 3H, s), 2.00 (3H, s, OAc), 3.78 (1H, dd, J=2, 5 Hz, H-3), 4.87 (1H, dd, J=10, 2 Hz, H-2). MS *m/e*: 282 (M^+). *Anal.* Calcd. for $C_{17}H_{30}O_3$: C, 72.30; H, 10.71. Found: C, 72.21; H, 10.84.

Ethanolysis of Buddledin A——To a solution of buddledin A (160 mg) in absolute EtOH (16 ml) was added 1% NaOEt–EtOH (1 ml) and the mixture was left standing at room temperature for 3 hr. The solution was neutralized with an ion-exchange resin (Amberlite IR-120) and filtered. The filtrate was evaporated to give a pale yellow syrup, which was purified by prep. TLC (solvent I) to yield ethyl ether (**4**) (87 mg) and deacetylbuddledin A (15 mg), mp 138—140°, $[\alpha]_D$ −310° (*c*, 0.59), which was identified with buddledin B (mixed mp, IR, UV, PMR, MS). Ethyl ether (**4**) was recrystallized from EtOH–H_2O to give colorless plates, mp 97—98°. $[\alpha]_D$ +73.4° (*c*, 1.1). IR ν_{max}^{KBr} cm^{-1}: 3450, 1695, 1634, 890. PMR δ: 1.09 (3H, d, J=7 Hz), 1.19 (3H, t, J=7 Hz), 1.13, 1.25 (each 3H, s), 3.50 (2H, q, J=7 Hz), 4.05 (1H, m, H-5), 4.35 (1H, d, J=12 Hz, H-2), 4.53 (1H, t, J=2 Hz, H-15), 4.69 (1H, br. s, H-15′). MS *m/e*: 280 (M^+). *Anal.* Calcd. for $C_{17}H_{28}O_3$: C, 72.82; H, 10.06. Found: C, 72.44; H, 10.15.

Reduction of Buddledin A with $LiAlH_4$——A solution of buddledin A (100 mg) in ether (20 ml) was added to a stirred suspension of $LiAlH_4$ (200 mg) in ether (20 ml) and the mixture was refluxed for 2 hr. After decomposition of the excess reagent with AcOEt, satd. aq. Na_2SO_4 was added to the reaction mixture to give a white precipitate. The supernatant liquor was decanted, washed with H_2O, and evaporated to yield a mixture of diols, (**5**) and (**6**), and deacetyldihydro derivative (**7**), which were fractionated by prep. TLC (solvent I).

Diol (**5**): *Rf* 0.22. mp 123—125° (from ether–petr. ether, 37 mg). $[\alpha]_D$ +43.8° (*c*, 0.96). IR ν_{max}^{KBr} cm^{-1}: 3420, 3250, 1630, 922, 880. PMR δ: 1.11, 1.19 (each 3H, s), 1.62 (3H, s, H-14), 3.75 [1H, m, converted into double doublet (J=3, 10 Hz) on addition of D_2O, H-2] 4.15 (1H, d, J=3 Hz, H-3), 4.90, 5.00 (each 1H, s, H-15), 5.87 (1H, m, H-5). MS *m/e*: 236 (M^+). *Anal.* Calcd. for $C_{15}H_{24}O_2$: C, 76.22; H, 10.24. Found: C, 76.03; H, 10.25.

Diol (**6**): *Rf* 0.16. mp 70—72° (from MeOH–H_2O, 14 mg). $[\alpha]_D$ −7.5° (*c*, 0.93). IR ν_{max}^{KBr} cm^{-1}: 3400, 3250, 1630, 990, 897. PMR δ: 1.14, 1.19 (each 3H, s), 1.65 (3H, s, H-14), 3.50 (1H, d, J=9 Hz, H-3), 3.74 (1H, t, J=9 Hz, H-2), 4.90, 5.03 (each 1H, s, H-15), 5.53 (1H, m, H-5). MS *m/e*: 236 (M^+). *Anal.* Calcd. for $C_{15}H_{24}O_2 \cdot 3/4H_2O$: C, 72.10; H, 10.29. Found: C, 72.34; H, 10.00.

Deacetyldihydrobuddledin A (**7**): *Rf* 0.51. Colorless syrup (6 mg). $[\alpha]_D$ −84.9° (*c*, 0.46). IR ν_{max}^{KBr} cm^{-1}: 3400, 3250, 1702, 1632, 898. PMR δ: 1.05 (3H, d, J=6 Hz, H-14), 1.16, 1.27 (each 3H, s), 4.20 (1H, d, J=11 Hz, H-2), 4.77 (2H, s, H-15). MS *m/e*: 236 (M^+). *Anal.* Calcd. for $C_{15}H_{24}O_2 \cdot 1/2H_2O$: C, 73.46; H, 10.20. Found: C, 73.33; H, 10.17.

Preparation of Bromohydrin (8)——A mixture of N-bromoacetamide (128 mg) and NaOAc (128 mg) in H_2O (3.8 ml) was added to a solution of buddledin A (100 mg) in acetone (10 ml) at 50°. After a few minutes, the reaction mixture was concentrated, diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was dried and evaporated to give a colorless syrup, which was separated by prep. TLC (solvent I) to give **1** (70 mg) and bromohydrin (**8**) (27 mg). Recrystallization of **8** from acetone–petr. ether provided colorless plates, mp 185—186°. $[\alpha]_D$ −73° (*c*, 0.57). IR ν_{max}^{KBr} cm^{-1}: 3520, 1736, 1678, 1655, 600. UV λ_{max} nm (ε): 237 (9700). PMR δ: 1.09 (6H, s), 1.81 (3H, s, H-14), 2.16 (3H, s, OAc), 3.11, 3.46 (2H, AB q, J=11 Hz, H-15), 5.73 (1H, d, J=11 Hz, H-2), 6.49 (1H, m, H-5). MS *m/e*: 330 (M^+−42), 332 [(M^++2)−42]. *Anal.*

Calcd. for $C_{17}H_{25}BrO_4$: C, 54.69; H, 6.75. Found: C, 54.40; H, 6.71. Acetylation of **8** with Ac_2O and pyridine resulted in recovery of the starting material.

X-Ray Analysis of Bromohydrin (8)——The intensity data and the cell dimensions were measured on a Syntex $P\bar{1}$ four-circle diffractometer using graphite monochromated Mo $K\alpha$ radiation ($\lambda=0.71069$ Å). Crystal data: $C_{17}H_{25}BrO_4$, mol. wt.=373.28, (*ortho*)rhombic, space group $p2_12_12_1$, $a=19.747$, $b=8.693$, $c=10.194$ Å, $V=1749.98$ Å^3, $Z=4$, $\rho_{calcd.}=1.417$ g cm^{-3}. Intensity data for $2\theta<50°$ were collected using the θ-2θ scanning technique. Of the total 1879 independent reflections, 1133 were found to be observed at a 2σ level of significance and used in the structure solution and refinement. During the data collection, three standard reflections were monitored every 97 measurements to check crystal alignment and the stability; no decrease in the intensity of standards was observed. The data were corrected for Lorenz and polarization factors, but no absorption correction was applied. The structure was solved by the heavy atom method and the positional and anisotropic thermal parameters for the non-hydrogen atoms were refined by the full-matrix least squares methods to a final R value of 0.078.[10] Hydrogen atoms were not included in the refinement. The final atomic coordinates with their estimated standard deviations are listed in Table I.[11]

Table I. Fractional Coordinates and Anisotropic Thermal Parameters[a] with Their Estimated Standard Deviations in Parentheses

Atom	X	Y	Z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
Br	0.0160(1)	0.0735(3)	1.1144(2)	21(0)	281(5)	180(3)	−29(1)	11(1)	15(4)
C (1)	0.2331(6)	0.2917(15)	0.9457(13)	21(4)	42(20)	63(15)	7(8)	0(7)	6(16)
C (2)	0.2402(5)	0.4549(12)	0.8859(14)	15(3)	29(19)	87(16)	−4(6)	−6(7)	21(18)
C (3)	0.1846(6)	0.5624(15)	0.9326(11)	22(4)	65(21)	38(13)	−3(8)	0(6)	16(17)
C (4)	0.1133(7)	0.5171(15)	0.9166(16)	19(4)	67(21)	127(23)	2(8)	−6(8)	23(20)
C (5)	0.1005(7)	0.4157(16)	0.8179(14)	22(4)	58(20)	105(19)	8(9)	−14(8)	6(21)
C (6)	0.0384(7)	0.3197(15)	0.8127(16)	24(4)	46(20)	139(22)	−4(8)	−31(8)	24(19)
C (7)	0.0653(7)	0.1517(17)	0.8048(16)	16(4)	103(26)	126(23)	−8(9)	−22(8)	−11(22)
C (8)	0.1188(6)	0.1033(15)	0.9115(15)	14(3)	85(22)	112(21)	0(7)	−14(7)	36(19)
C (9)	0.1944(6)	0.1554(14)	0.8754(15)	15(3)	74(20)	91(17)	4(7)	−9(8)	10(19)
C (10)	0.2494(6)	0.0453(15)	0.9297(16)	18(4)	57(23)	148(24)	6(8)	−9(8)	7(20)
C (11)	0.3001(7)	0.1880(15)	0.9397(14)	19(4)	62(20)	82(17)	13(8)	0(7)	31(18)
C (12)	0.3438(7)	0.1901(21)	1.0622(17)	21(4)	172(32)	130(24)	0(11)	−15(9)	16(25)
C (13)	0.3414(8)	0.2005(19)	0.8122(17)	34(6)	110(28)	119(23)	26(12)	23(10)	−27(23)
C (14)	0.0635(7)	0.5824(18)	1.0180(18)	28(5)	54(20)	175(26)	5(10)	31(9)	−15(24)
C (15)	0.1040(6)	0.1510(18)	1.0539(14)	10(3)	170(30)	91(18)	−21(9)	12(7)	18(20)
C (16)	0.3282(6)	0.6326(15)	0.8671(16)	17(4)	68(21)	115(21)	−10(7)	9(9)	24(21)
C (17)	0.3857(7)	0.7138(21)	0.9463(18)	14(4)	181(33)	188(29)	−10(10)	−9(9)	2(27)
O (1)	0.3032(4)	0.5139(10)	0.9326(10)	18(3)	93(16)	102(13)	−9(6)	−5(5)	11(13)
O (2)	0.3040(7)	0.6807(14)	0.7655(11)	53(5)	171(23)	103(15)	−42(10)	−3(8)	60(18)
O (3)	0.2018(5)	0.6812(11)	0.9968(11)	25(3)	111(17)	125(15)	−1(7)	1(6)	−37(16)
O (4)	0.1176(6)	−0.0642(10)	0.8970(12)	28(3)	49(13)	160(15)	−7(6)	−6(7)	−13(16)

a) Anisotropic temperature factors ($\times10^4$) are of the form $\exp[-(\beta_{11}h^2+\beta_{22}k^2+\beta_{33}l^2+2\beta_{12}hk+2\beta_{13}hl+2\beta_{23}kl)]$.

Reduction of Tetrahydrobuddledin A (3) with $NaBH_4$——Tetrahydrobuddledin A (380 mg) in dioxane (40 ml) was reduced with $NaBH_4$ (760 mg) in H_2O (2 ml) for 12 hr at room temperature. The solvent was removed, water added, and the solution extracted with $CHCl_3$. Evaporation of the dried solvent gave a colorless syrup which was purified by prep. TLC (solvent I). The band of Rf 0.54 gave the unreacted **3** (115 mg). The fraction of Rf 0.22 (94 mg) was revealed by PMR spectrum to be a mixture of 3-epihexahydrobuddledin A, 2-hydroxy-3-*O*-acetyltetrahydrobuddledin A and **9** (3:4:5). Repeated recrystallization of the mixture from MeOH–H_2O gave colorless needles (25 mg) identical with hexahydrobuddledin A (**9**) prepared by catalytic hydrogenation of **1**.

Mesylation of 9——Methanesulfonyl chloride (1 ml) was added to a solution of **9** (70 mg) in pyridine (0.8 ml). The reaction mixture was allowed to stand at room temperature for 20 min and poured into ice-water, and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with 1 N HCl, 10% NaOH and satd. aq.

10) The computer programs used are given in "UNICS" by T. Sakurai, 1967. The atomic scattering factors used in these calculations were taken from "International Tables for X-ray Crystallography," Vol. III, Kynoch Press, Birmingham, England, 1965.

11) Table for the observed and calculated structure factors may be obtained from the authors.

NaCl, dried and evaporated. The crude product was purified by prep. TLC (solvent I) to yield syrupy mesylate (**10**) (46 mg). $[\alpha]_D$ +4.7° (*c*, 1.45). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1726, 1241—1208, 1171, 1020, 908. PMR δ: 0.95 (3H, d, J=6 Hz, H-15), 1.00, 1.06 (each 3H, s), 1.08 (3H, d, J=6 Hz, H-14), 2.04 (3H, s, OAc), 3.06 (3H, s, CH_3-SO_2-), 4.82 (1H, dd, J=2, 4 Hz, H-3), 5.01 (1H, dd, J=10, 2 Hz, H-2). MS *m/e*: 360 (M^+).

Reduction of 10 with $LiAlH_4$——To a stirred suspension of $LiAlH_4$ (96 mg) in dry ether (10 ml) was added **10** (46 mg) in dry ether (5 ml) at 0°, and the mixture was refluxed for 2 hr. The reaction was quenched by careful addition of AcOEt and satd. aq. Na_2SO_4. The supernatant liquor was decanted and washed with H_2O, dried and evaporated to give a colorless syrup (33 mg). Purification by prep. TLC (solvent I) followed by recrystallization from MeOH–H_2O gave **11** (11 mg), mp 79—81°. $[\alpha]_D$ −37.9° (*c*, 0.60). IR ν_{max}^{KBr} cm^{-1}: 3280, 1620. PMR ($CDCl_3$+D_2O) δ: 0.85 (3H, d, J=6 Hz), 1.08, 1.21 (each 3H, s), 1.56 (3H, d, J=1 Hz, H-14), 4.40 (1H, dd, J=11, 6 Hz, H-2), 5.27 (1H, dd, J=1, 6 Hz, H-3). MS *m/e*: 222 (M^+). *Anal.* Calcd. for $C_{15}H_{26}O \cdot 1/4H_2O$: C, 79.41; H, 11.77. Found: C, 79.61; H, 11.67.

Catalytic Hydrogenation of 11——A mixture of **11** (9 mg) and preactivated PtO_2 (9 mg) in AcOH (5 ml) was stirred under atmospheric pressure of H_2 for 2 hr at 37—40°. The catalyst was filtered off and the filtrate was evaporated to give the saturated monoalcohol (**12**) (8.5 mg), mp 51—52° (from MeOH–H_2O). $[\alpha]_D$ +5.9° (*c*, 0.85). IR ν_{max}^{KBr} cm^{-1}: 3300. PMR δ: 0.94 (6H, d, J=6 Hz, H-14 and 15), 1.09, 1.16 (each 3H, s), 3.64 (1H, m, H-2). MS *m/e*: 224 (M^+). *Anal.* Calcd. for $C_{15}H_{28}O \cdot 1/2H_2O$: C, 77.25; H, 12.44. Found: C, 77.41; H, 12.21.

Benzoylation of 12——The alcohol (**12**) (8.5 mg) was benzoylated with benzoyl chloride (0.05 ml) in pyridine (0.5 ml) at room temperature for 2 hr. The usual work-up gave a crude product, which was purified by prep. TLC (solvent I) to yield the benzoate (**13**) (7.5 mg) as colorless syrup. $[\alpha]_D$ +63.3° (*c*, 0.73). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1703, 1620, 1275. PMR δ: 0.99, 1.00 (each 3H, d, J=6 Hz, H-15 and 14), 0.99, 1.09 (each 3H, s), 5.00 (1H, m, H-2), 7.53, 8.10 (5H, arom. H). MS *m/e*: 328 (M^+). *Anal.* Calcd. for $C_{22}H_{32}O_2 \cdot 1/4H_2O$: C, 79.39; H, 9.69. Found: C, 79.23; H, 9.33.

Benzoylation of 5——A mixture of **5** (20 mg), benzoyl chloride (0.05 ml) and pyridine (0.5 ml) was allowed to stand overnight at room temperature and then poured into ice-water. The resultant solution was extracted with $CHCl_3$, and the $CHCl_3$ extract was washed with 10% HCl, 10% NaOH and satd. aq. NaCl, dried and evaporated to give a pale yellow syrup. The product was purified by passing through Al_2O_3 column to afford crystalline benzoate. Recrystallization from MeOH–H_2O gave **14** as colorless fine needles (16 mg), mp 129—130°. $[\alpha]_D$ +139° (*c*, 0.24). IR ν_{max}^{KBr} cm^{-1}: 1715, 1705 (shoulder), 1598, 1580, 883, 708. UV λ_{max} nm (ε): 228 (26000), 273 (1900), 281 (1700). PMR δ: 0.99, 1.17 (each 3H, s), 1.87 (br. s, H-14), 4.97 (1H, s, H-15), 5.12 (1H, br. s, H-15'), 5.36 (1H, dd, J=10, 3 Hz, H-2), 5.67 (1H, d, J=3 Hz, H-3), 5.80 (1H, m, H-5), 7.92 (4H, m), 7.43 (6H, m) (arom. H). MS *m/e*: 444 (M^+). *Anal.* Calcd. for $C_{29}H_{32}O_4 \cdot 1/4H_2O$: C, 77.56; H, 7.19. Found: C, 77.64; H, 7.27.

Benzoylation of 6——To a solution of **6** (13 mg) in pyridine (0.5 ml) was added benzoyl chloride (0.05 ml). The reaction mixture was worked up in an analogous way to the preparation of **14** to give **15** as colorless needles, mp 122—123° (from MeOH–H_2O). $[\alpha]_D$ −10.9° (*c*, 0.46). IR ν_{max}^{KBr} cm^{-1}: 1730, 1711, 1624, 1603, 1583, 892, 706. UV λ_{max} nm (ε): 228 (32000), 275 (1900), 280 (1600). PMR δ: 1.04, 1.11 (each 3H, s), 1.68 (3H, br.s, H-14), 5.02, 4.91 (each 1H, br.s, H-15), 5.31 (1H, d, J=10 Hz, H-3), 5.64 (1H, t, J=10 Hz, H-2), 5.78 (1H, m, H-5), 7.33 (4H, m), 7.91 (6H, m) (arom. H). MS *m/e*: 444 (M^+). *Anal.* Calcd. for $C_{29}H_{32}O_4 \cdot 1/4H_2O$: C, 77.56; H, 7.19. Found: C, 77.30; H, 7.18.

Reduction of 3 with $LiAlH_4$——A solution of **3** (120 mg) in dry ether (10 ml) was added dropwise to a stirred suspension of $LiAlH_4$ (200 mg) in dry ether (30 ml) at 0°. After refluxing for 2 hr, the reaction mixture was worked up in an analogous way to the preparation of the diols (**5**) and (**6**), to give a crude product (89 mg), which was recrystallized from *n*-hexane to yield **16** (67 mg) as colorless needles, mp 82—84°. $[\alpha]_D$ +12.6° (*c*, 1.03). IR ν_{max}^{KBr} cm^{-1}: 3500, 3340, 1458, 1383. PMR (C_5D_5N–D_2O) δ: 0.97, 1.14 (each 3H, d, J=7 Hz, H-15, 14), 1.26, 1.40 (each 3H, s), 2.44 (1H, t, J=10 Hz, H-1), 3.86 (1H, dd, J=10, 3 Hz, H-2), 4.03 (1H, t, J=3 Hz, H-3). MS *m/e*: 240 (M^+). *Anal.* Calcd. for $C_{15}H_{28}O_2$: C, 74.95; H, 11.74. Found: C, 74.97; H, 12.04.

Benzoylation of 16——The diol (**16**) (27 mg) was benzoylated with benzoyl chloride (0.05 ml) and pyridine (0.5 ml) at room temperature overnight. The usual work-up gave the benzoate (**17**) (13 mg) as colorless syrup. $[\alpha]_D$ +44.4° (*c*, 1.35). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1713, 1450, 1225, 1213. UV λ_{max} nm (ε): 228 (24000), 272 (1300), 281 (1300). PMR (CCl_4) δ: 1.00, 1.04 (each 3H, s), 1.04, 1.12 (each 3H, d, J=6 Hz, H-15, 14), 5.42 (2H, m, H-2, 3), 7.44, 7.96 (10H, arom. H). MS *m/e*: 448 (M^+).

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