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Studies on the Constituents of *Buddleja* Species. II.[1] Buddledin C, D and E, New Sesquiterpenes from *Buddleja davidii* Franch.[2]

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Further toxic principle, buddledin C, and two additional sesquiterpenes, buddledin D and E, have been isolated from the root bark of *Buddleja davidii* Franch. The structures of buddledin C and D have been determined to be **3** and **4** by chemical transformation of buddledin A into buddledin D and dihydrobuddledin C (**6**), and by comparisons of their spectral data with those of buddledin A and its *cis*-isomer (**8**), which was obtained by the photoisomerization of **1**. Structure **5** has been assigned for buddledin E by its spectral data and deuteration experiment.

Keywords——*Buddleja davidii* Franch.; Buddlejaceae; sesquiterpene of caryophyllene skeleton; buddledin C; buddledin D; buddledin E; PMR and CMR spectra; photoisomerization; piscicidal activity

In the preceding paper[1] we reported on the structural elucidation of two toxic sesquiterpenes, buddledin A (**1**) and B (**2**) isolated from the root bark of *Buddleja davidii* Franch. (Buddlejaceae). Further examination of this plant has led to the isolation of another toxic

Table I. Physical and Spectral Data of Buddledin A (**1**), C (**3**) and D(**4**)

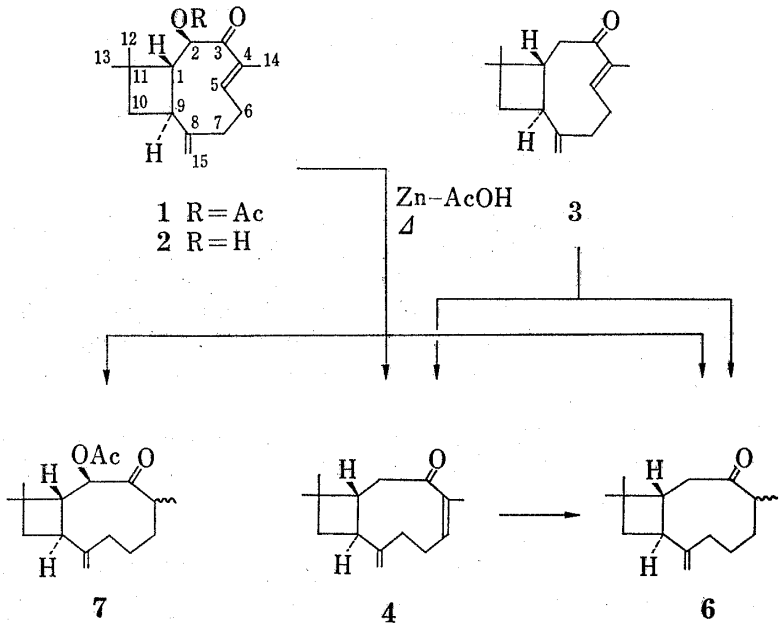
	1	**3**	**4**
mp	94—95°	129—130°	Syrup
Formula	$C_{17}H_{24}O_3$	$C_{15}H_{22}O$	$C_{15}H_{22}O\cdot 1/4H_2O$
Anal. (%)			
Calcd.	*a*)	C, 82.51 H, 10.16	C, 80.85 H, 10.17
Found	*a*)	C, 82.26 H, 10.10	C, 81.09 H, 10.21
MS *m/e* (M^+)	276	218	218
$[\alpha]_D^{CHCl_3}$ (*c*)	−245° (0.74)	−316° (0.54)	−164° (0.92)
IR ν_{max}^{KBr} cm^{-1}	1742, 1682, 1642, 890	1662 (br.) 887	1665 (br.) 880
PMR ($CDCl_3$) δ:*b*)			
H-2	5.67(d, 11)	*c*)	*c*)
H-12, 13	1.11(s)	1.00(s)	1.02(s)
($C\underline{H}_3$)	1.10(s)		1.01(s)
H-15	4.93(s)	4.92(s)	4.88(s)
(=$C\underline{H}_2$)		4.95(s)	4.82(br. s)
OAc	2.14	—	—

a) See ref. 1
b) Multiplicities and coupling constants (Hz) are given in parentheses. The data for H-5 and H-14 are given in Table II.
c) Overlapped by other protons at δ 3.0—1.5.

1) Part I: T. Yoshida, J. Nobuhara, M. Uchida, and T. Okuda, *Chem. Pharm. Bull.* (Tokyo), **26**, 2535 (1978).
2) A part of this work was reported in a short preliminary communication: T. Yoshida, J. Nobuhara, M. Uchida, and T. Okuda, *Tetrahedron Lett.*, **1976**, 3717.
3) Location: *1-1-1, Tsushima-naka, Okayama 700, Japan.*

principle, named buddledin C (**3**), and two additional sesquiterpenes, buddledin D (**4**) and E (**5**).

Physical and spectral data of buddledin C and D together with those of buddledin A are listed in Table I and II.



Scheme 1

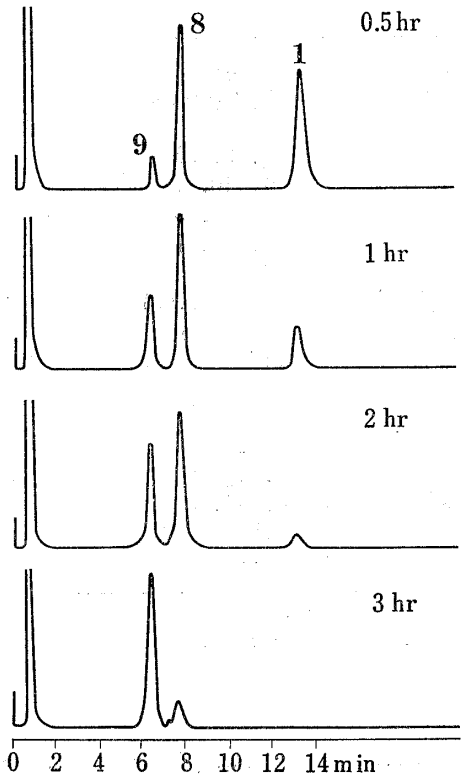


Fig. 1. Gas Chromatograms of Photoisomerized Products from **1**

2.5% OV-17, column temperature, 180°.

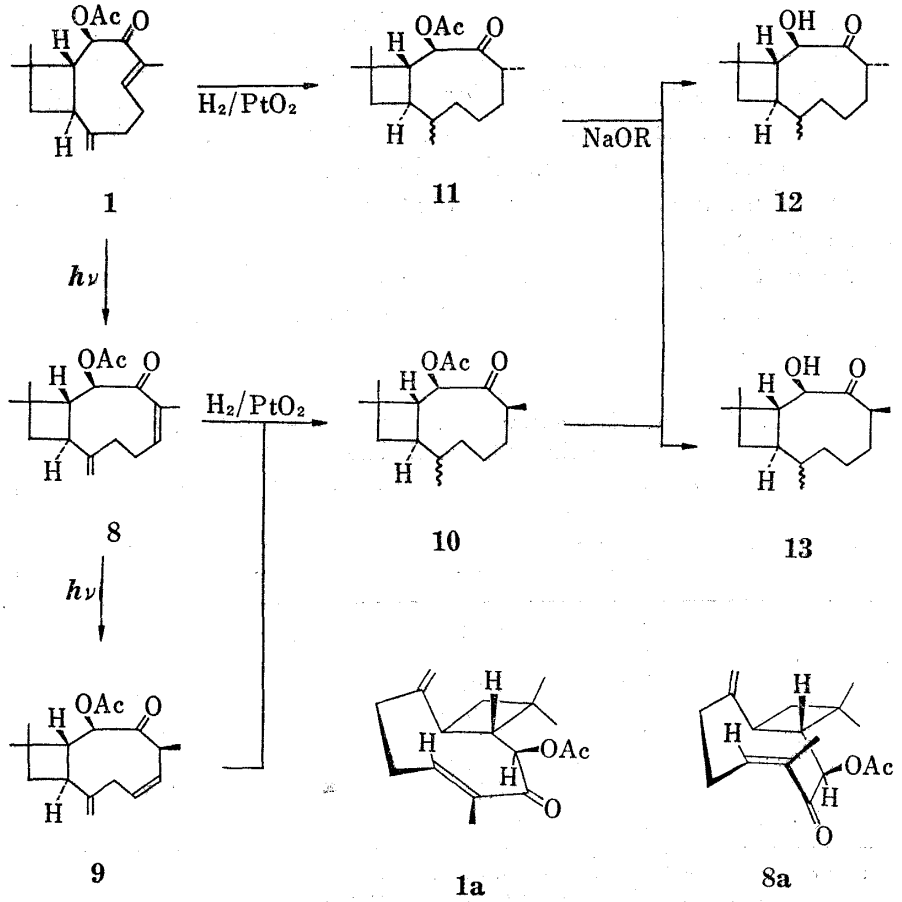
The spectroscopic properties of buddledin C and D are similar to those of **1** except for those attributable to the acetoxyl group. The relationship among buddledin A, C and D has been shown in the following way.

Buddledin A was converted upon treatment with Zn–AcOH to deacetyldihydrobuddledin A (**6**), $C_{15}H_{24}O$ and dihydrobuddledin A (**7**), $C_{17}H_{26}O_3$. The former was identified with the product (dihydrobuddledin C) obtained by the same treatment of buddledin C. Treatment of buddledin C with Zn in milder conditions gave buddledin D in addition to **6**. Buddledin D, along with **7** and a small amount of **6**, were also obtained by analogous reaction of **1**. These findings establish that buddledin C and D have the same carbon skeleton including the position of ketone group as that of buddledin A, and also that they are regarded as differing only in geometry about the endocyclic double bond.

The distinguishable feature between buddledin C and D in the proton nuclear magnetic resonance (PMR) spectra is the multiplet assignable to an olefinic proton of α,β-unsaturated ketone group: it is shown at δ 6.33 in buddledin C, which is similar to that (δ 6.45) of buddledin A, while the corresponding multiplet in buddledin D shows a higher-field shift

(δ 5.53) than that expected for the β-proton of such a system. Since the *trans* endocyclic double bond in caryophyllene is known to be less stable than *cis* double bond,[4] the isomerization of buddledin C to buddledin D, coupled with the analogy of the chemical shift of H–5 in buddledin C to that of buddledin A, suggest that the geometry of the concerned double bond is *trans* in buddledin C and *cis* in buddledin D. This assumption has been supported by the comparisons of their spectral data with those of buddledin A and its *cis*-isomer as follows. Buddledin A, whose endocyclic double bond was established to be *trans* by X-ray analysis of bromohydrin,[1] was isomerized upon irradiation with low pressure mercury lamp to isomer-I (**8**), $C_{17}H_{24}O_3$, which was further isomerized under prolonged irradiation to give isomer-II (**9**), $C_{17}H_{24}O_3$, mp 69—70°. The stepwise isomerization was exhibited by the gas-liquid chromatography (GLC) as shown in Fig. 1.

Presence of a conjugated ketone group in **8** is shown in the ultraviolet (UV) spectrum (237 nm, ε 3400), while the endocyclic double bond of **9**, which is disubstituted (PMR, δ 5.53, 2H, m), is not conjugated with the carbonyl group (no UV absorption around 230 nm). The carbon skeleton of **1** has been confirmed to be retained during the photoreaction as follows. Both **8** and **9** gave, upon catalytic hydrogenation, the same tetrahydro derivative (**10**), $C_{17}H_{28}O_3$, mp 63—64°, which, however, was different from tetrahydrobuddledin A (**11**)[1] obtainable by catalytic hydrogenation of **1**. The PMR spectrum of **10** is similar to that of **11**, but the signal of C_4-methyl group (δ 1.22, d, J=7 Hz) shows a lower-field shift than that in **11** (δ 1.11, d, J=7 Hz). As the tetrahydro derivative (**10**) is regarded as the epimer of **11** concerning C_4-methyl group, establishment of correlation between these two compounds



Scheme 2

4) A. Aebi, D.H.R. Barton and A.S. Lindsey, *J. Chem. Soc.*, **1953**, 3124.

were attempted by epimerization at C_4. Alcoholysis of **11** yielded an epimeric mixture of deacetyl derivatives, which was also produced by an analogous treatment of **10**. Both of these mixtures gave **12**, $C_{15}H_{26}O_2$, mp 45—47° and **13**, $C_{15}H_{26}O_2$, mp 72—73° upon separation by preparative thin-layer chromatography (prep. TLC), establishing the retention of the basic carbon skeleton of **1** in **8** and **9**.

These findings indicate that isomer-I is *cis*-isomer (**8**) of buddledin A and isomer-II is β,γ-unsaturated ketone (**9**).

It is assumed that the production of epimeric tetrahydro derivatives upon hydrogenation of **1** and **8** is due to their conformational difference. Buddledin A is presumed to have the conformation **1a** based on the spectral similarity to that of the bromohydrin which was elucidated by X-ray analysis.[1] This conformation **1a** is considered to permit hydrogen transfer to β-face of the endocyclic double bond to give **11** which has α-oriented methyl group. On the other hand, the non-planarity of conjugated system in **8a**, as shown by the spectral data described later, is considered to allow preferencial hydrogenation at the less hindered α-face, to afford **10** having β-oriented methyl group at C_4. The C_4-methyl group in **9** is, therefore, also regarded as β-oriented. The configurations at C_8 in **10**—**13** are unknown. However, the results described above, combined with the PMR spectra show that the configurations of C_8-methyl group in these compounds are the same.

Upon isomerization of **1** to **8**, significant changes in the spectroscopic properties have been observed. The PMR spectrum of **8** shows an upfield shift of H-5 signal by 0.85 ppm (δ 6.45→5.60) and a downfield shift of the vinyl methyl signal (δ 1.64→2.01), from those of **1**. The ^{13}C nuclear magnetic resonance (CMR) spectrum also shows upfield shift of C_5 carbon and downfield shift of carbonyl carbon (Table II). These observations, coupled with the smaller extinction coefficient in the UV spectrum of **8**, indicate that the electron distribution in the α,β-unsaturated ketone system in **8** differs from that in **1**. The upfield shifts of C-5 and H-5 in **8** are attributable to the smaller contribution of resonance form due to significant departure from coplanarity of the ketone group and the double bond.[5] The weak intensity in the UV spectrum of **8** is also attributable to the distortion of the conjugated system.[6] The downfield shift of the vinyl methyl signal is ascribable to the 1,3-diaxial-like arrangement between C_4-Me and C_2-OAc group in **8a**.

The difference of the spectral data between buddledin C and D (Table II) is analogous to that between buddledin A and its *cis*-isomer, to indicate that their structures are represented by **3** and **4**, respectively.

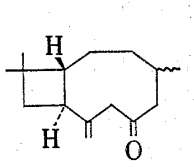
Table II. UV and NMR Spectra[a] of Buddledin A (**1**), Its *cis*-Isomer (**8**), Buddledin C (**3**) and Buddledin D (**4**)

	UV λ_{max}^{MeOH} nm (ε)	PMR		CMR		
		H-5	H-14	C-3	C-4[b]	C-5[b]
1	238 (8500)	6.45 (m)	1.64 (br. s)	197.3	134.6	142.4
8	237 (3400)	5.60 (m)	2.01 (br. s)	204.8	138.4	132.3
3	236 (11000)	6.33 (m)	1.63 (br. s)	206.3	136.3	143.5
4	235 (4700)	5.53 (m)	1.78 (br. s)	209.1	137.2	132.3

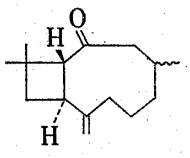
a) Spectra were taken in $CDCl_3$, and chemical shifts are given in δ (ppm).
b) Assigned by the multiplicities in proton off-resonance decoupled spectra.

5) J.B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, New York, N.Y., 1972. pp. 280, 437.
6) J. Wolinsky and D. Chan, *J. Am. Chem. Soc.*, **85**, 937 (1963).

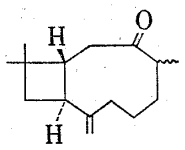
Buddledin E, $C_{15}H_{24}O$ (M^+ 220), $[\alpha]_D$ −6° (*c*, 1.33, $CHCl_3$), shows IR band at 1690 cm^{-1} ($CHCl_3$), but no absorption of conjugated ketone is observed in the UV spectrum. The PMR spectrum exhibits signals of exomethylene group at δ 4.69 (t, J=2 Hz) and 4.82 (br. s), and a secondary methyl signal at δ 1.03 (d, J=6 Hz), in addition to a signal (δ 1.06, 6H, s) of two tertiary methyl groups. These spectral data indicate that one of the structures, **5** and **5a**—**5c**, is assignable to buddledin E, among which **5** is regarded as the most plausible one by production of d_4-derivative, which was analyzed by combined gas chromatography–mass spectrometry (GC-MS), upon deuteration of buddledin E with 2 N DCl in dioxane.



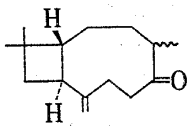
5



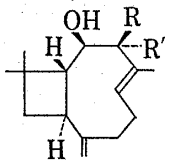
5a



5b



5c



14 : R=OH, R′=H
15 : R=H, R′=OH

Chart 1

Buddledin C which shows toxicity (median tolerance limit after 24 hr, 1.6 ppm) on the killie-fish bioassay[1] is the third toxic sesquiterpene in this plant. Buddledin D and E, and also dihydrobuddledin A (**7**), tetrahydrobuddledin A (**11**), dihydrobuddledin C (**6**), diols, (**14**)[1] and (**15**),[1] however, showed no toxicity to killie-fish. Comparisons of these structures indicate that the ketone group at C_3 conjugated with the *trans* double bond is essential for the toxicity.

Experimental

GLC was carried out with Shimadzu 5A gas chromatograph equipped with hydrogen flame ionization detector (FID) using glass column (2 m×3 mm i.d.) packed with 2.5% OV-17 on 80—100 mesh Chromosorb W. CMR spectra were measured in $CDCl_3$ with JEOL FX 60 at 15 MHz with ^2H internal lock and tetramethylsilane as the internal standard. Solvent systems for TLC and prep. TLC: solvent I, benzene–AcOEt (9:1); solvent II, benzene–AcOEt (98:2); solvent III, $CHCl_3$–*n*-hexane (1:1); solvent IV, ether–*n*-hexane (2:3). Other instruments used in the experimental section were same as in the preceding paper.[1]

Isolation of Buddledin C, D and E——The fraction of *Rf* 0.54 (TLC, solvent III), which was obtained by the column chromatography and prep. TLC (solvent III) of the ethereal extract of *Buddleja davidii* root bark,[1] was further fractionated by prep. TLC developed with solvent II to give buddledin C (*Rf* 0.39, 390 mg) as colorless prisms, and a mixture of buddledin D and E (*Rf* 0.41) as syrups. This mixture was separated into its components by prep. TLC over 10% $AgNO_3$–silica gel (solvent IV) to yield buddledin D (*Rf* 0.59, 144 mg) and buddledin E (*Rf* 0.64, 86 mg), respectively. Physical and spectral data of these compounds are shown in the text.

Zinc Reduction of Buddledin A——(i) To a solution of buddledin A (200 mg) in AcOH (4 ml) was added Zn powder (1 g), and the mixture was refluxed for 1.5 hr. After cooling, the reaction mixture was diluted with AcOEt (100 ml), filtered, and the filtrate was washed with satd. aq. $NaHCO_3$ and satd. aq. NaCl. The organic phase was dried and evaporated to give a colorless syrup which was separated by prep. TLC (solvent II) into **6** (*Rf* 0.57, 28 mg) and **7** (*Rf* 0.46, 29 mg).

Deacetoxydihydrobuddledin A (**6**): colorless syrup. $[\alpha]_D$ +42.6° (*c*, 0.27, dioxane). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1692, 1632, 1455, 895. PMR δ: 1.04 (6H, s), 1.06 (3H, d, J=6 Hz, H-14), 4.54 (1H, t, J=2 Hz, H-15), 4.70 (1H, s, H-15′). MS *m/e*: 220 (M^+). *Anal.* Calcd. for $C_{15}H_{24}O \cdot 1/3H_2O$: C, 79.64; H, 10.76. Found: C, 79.49; H, 10.86.

Dihydrobuddledin A (**7**): colorless syrup. $[\alpha]_D$ +119.5° (*c*, 0.59). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720, 1632, 1460, 1369, 1245, 897. PMR δ: 1.09 (3H, d, J=6 Hz, H-14), 1.11, 1.13 (each 3H, s), 2.06 (3H, s, OAc), 4.61 (1H, t, J=2 Hz, H-15), 4.76 (1H, br.s, H-15'), 5.12 (1H, d, J=12 Hz, H-2). MS *m/e*: 278 (M^+). *Anal.* Calcd. for $C_{17}H_{26}O_3$: C, 73.34; H, 9.41. Found: C, 72.96; H, 9.33.

(ii) To a stirred solution of buddledin A (200 mg) in AcOH (10 ml), Zn powder (500 mg) was added, and the mixture was refluxed for 20 min. The reaction mixture was worked up in a similar way to (i) to yield **7** (*Rf* 0.46, 10 mg), unreacted **1** (*Rf* 0.35, 49 mg) and a syrup (*Rf* 0.57, 48 mg). This syrup was further purified by prep. TLC (20% $AgNO_3$-silica gel, solvent IV) to give a small amount of **6** (*Rf* 0.75) and a colorless syrup (*Rf* 0.63, 38 mg), $[\alpha]_D$ −199° (*c*, 0.7), which was identified with buddledin D (**4**) (MS, IR, UV, PMR).

Zinc Reduction of Buddledin C——(i) Zinc powder (300 mg) was added to a solution of buddledin C (59 mg) in AcOH (4 ml). The mixture was refluxed for 2 hr, diluted with AcOEt, and filtered. The filtrate was washed with 10% $NaHCO_3$ and satd. aq. NaCl. Evaporation of the dried solvent gave a colorless syrup which was purified by prep. TLC (solvent II) to afford dihydrobuddledin C (17.5 mg), $[\alpha]_D$ +41.7° (*c*, 0.3, dioxane) as a colorless syrup, which was identified with deacetyldihydrobuddledin A (**6**) (IR, MS, PMR).

(ii) To a solution of buddledin C (60 mg) in AcOH (10 ml) was added Zn powder (100 mg), and the mixture was refluxed for 25 min. Work-up analogous to (i) gave a crude product which was purified by prep. TLC (20% $AgNO_3$–silica gel, solvent IV) to afford **6** (*Rf* 0.75, 15 mg) and buddledin D (*Rf* 0.63) contaminated by a small amount of **3**. The latter (20 mg) was further purified by prep. TLC (solvent II) to give pure buddledin D (11 mg).

Photoisomerization of Buddledin A——A solution of buddledin A (50 mg) in hexane (20 ml) was perfused with N_2 for 5 min in a quartz tube and then irradiated with low pressure mercury lamp, being monitored by GLC. The irradiation was stopped after 75 min and the solution was evaporated and the residue was purified by prep. TLC (solvent I) to give unchanged **1** (*Rf* 0.45, 6 mg) and syrupy *cis*-isomer (**8**) (*Rf* 0.51, 19 mg). $[\alpha]_D$ −209° (*c*, 0.7). IR ν_{max}^{KBr} cm^{-1}: 1738, 1707, 1668 (w), 1633 (w), 1236, 895. PMR δ: 1.08, 1.12 (each 3H, s), 2.01 (3H, s, H-14), 2.08 (3H, s, OAc), 4.85 (2H, s, H-15), 5.26 (1H, d, J=10 Hz, H-2), 5.60 (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, 73.42; H, 8.87. The UV spectrum is shown in Table II. Prolonged irradiation (3 hr) under same conditions afforded β,γ-unsaturated ketone (**9**) (*Rf* 0.56, 25 mg). mp 69—70°. $[\alpha]_D$ +2.5° (*c*, 0.2). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1734, 1709, 1640, 900. PMR δ: 1.07, 1.10 (each 3H, s), 1.23 (3H, d, J=6 Hz), 2.04 (3H, s, OAc), 4.69 (1H, br.s, H-15), 4.82 (1H, t, J=2 Hz, H-15'), 5.11 (1H, d, J=10 Hz, H-2), 5.53 (2H, m, H-5 and 6). MS *m/e*: 276 (M^+). *Anal.* Calcd. for $C_{17}H_{24}O_3 \cdot 1/4H_2O$: C, 72.69; H, 8.79. Found: C, 72.75; H, 8.53.

Hydrogenation of 8——*cis*-Isomer (**8**) (56 mg) in EtOH (10 ml) was hydrogenated over preactivated PtO_2 (10 mg) at room temperature under atmospheric pressure. The catalyst was filtered off and the solvent was evaporated. The crude product was purified by prep. TLC (solvent I) followed by recrystallization from MeOH–H_2O to give 4-epimer of tetrahydrobuddledin A (**10**) (*Rf* 0.57, 36 mg) as colorless fine needles, mp 63—64°. $[\alpha]_D$ −71° (*c*, 2.0). IR ν_{max}^{KBr} cm^{-1}: 1738, 1730, 1707, 1243. PMR δ: 0.81 (3H, d, J=6 Hz), 1.07, 1.10 (each 3H, s), 1.22 (3H, d, J=7 Hz), 2.04 (3H, s, OAc), 5.34 (1H, d, J=11 Hz, H-2). MS *m/e*: 280 (M^+). *Anal.* Calcd. for $C_{17}H_{28}O_3 \cdot 1/4H_2O$: C, 71.67; H, 10.08. Found: C, 71.64; H, 10.02.

Hydrogenation of 9——β,γ-Unsaturated ketone (**9**) (127 mg) in EtOH (13 ml) was hydrogenated over prereduced PtO_2 (30 mg) at room temperature and atmospheric pressure for 40 min. Catalyst was removed by filtration and the solvent was evaporated to give a crude product. Purification by prep. TLC (solvent I) and recrystallization from MeOH–H_2O yielded colorless needles, mp 64—65° (65 mg), which was identified with **10** (mixed mp, IR, PMR, MS, $[\alpha]_D$).

Ethanolysis of Tetrahydrobuddledin A (**11**)——To a solution of **11**[1] (64 mg) in EtOH (6 ml) was added 1% NaOEt in EtOH (2 ml). The mixture was allowed to stand overnight at room temperature and neutralized with Amberlite IR-120. The filtrate was evaporated to give a pale yellow oil which was purified by prep. TLC (solvent I). The band at *Rf* 0.48 gave deacetyltetrahydrobuddledin A (**12**) (37 mg) as colorless needles, mp 45—47° (from MeOH–H_2O). $[\alpha]_D$ −161.8° (*c*, 1.03). IR ν_{max}^{KBr} cm^{-1}: 3450, 1700, 1456, 1002. PMR δ: 0.84, 1.07 (each 3H, d, J=6 Hz), 1.11, 1.23 (each 3H, s), 2.90 (1H, d, J=8 Hz, OH), 4.31 [1H, dd, J=8, 10 Hz, changed to doublet (J=10 Hz) on addition of D_2O, H-2]. MS *m/e*: 238 (M^+). *Anal.* Calcd. for $C_{15}H_{26}O_2$: C, 75.58; H, 11.00. Found: C, 75.39; H, 11.06.

The band of *Rf* 0.34 gave epimer (**13**) of **12** (8 mg) as colorless needles, mp 72—73° (from MeOH–H_2O). $[\alpha]_D$ −121° (*c*, 0.58). IR ν_{max}^{KBr} cm^{-1}: 3450, 1693. PMR δ: 0.80 (3H, d, J=7 Hz), 1.13, 1.22 (each 3H, s), 1.16 (3H, d, J=6 Hz), 4.42 [1H, m, converted into doublet (J=10 Hz) on addition of D_2O, H-2]. MS *m/e*: 238 (M^+). *Anal.* Calcd. for $C_{15}H_{26}O_2 \cdot 1/3H_2O$: C, 73.66; H, 10.76. Found: C, 73.88; H, 10.52.

Methanolysis of 10——To a solution of **10** (44 mg) in MeOH (3 ml) was added 0.1% NaOMe in MeOH (0.5 ml) and the mixture was left standing at room temperature for 5 hr, and neutralized with Amberlite IR-120 and filtered. The filtrate was evaporated to give a colorless oil. Separation by prep. TLC (solvent I) yielded **12** (*Rf* 0.48, 6.5 mg) and **13** (*Rf* 0.34, 15.5 mg). Identities among these materials and the products derived from **11** were established by mp, mixed mp, IR, PMR and $[\alpha]_D$.

Deuteration of Buddledin E——Buddledin E (2 mg) was dissolved in 2 N DCl-dioxane (0.2 ml), which was prepared by adding D_2O (0.25 ml) to a mixture of acetyl chloride (0.75 g) and dioxane (2 ml). After 2.5 hr the mixture diluted with ether and washed with H_2O. Evaporation of the ethereal solution afforded

a syrupy residue, whose GC-MS (2.5% OV-210, column temperature, 110—130°, 3°/min, t_R 6.4 min) showed peaks at *m/e* 224 (30%, d_4), 223 (36%, d_3), 222 (17%, d_2), 221 (5%, d_1), 220 (2%, d_0).

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