Structure and Stereochemistry of Pulchellin B, C, E, and F[1,2]

H. YOSHIOKA AND T. J. MABRY

The Cell Research Insitute and Department of Botany, University of Texas at Austin, Austin, Texas 78712

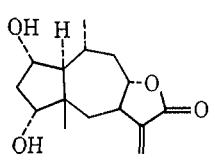
N. DENNIS AND WERNER HERZ[3]

Department of Chemistry, The Florida State University, Tallahassee, Florida 32306

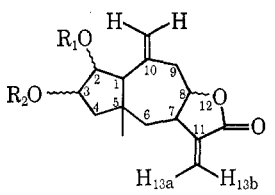
Received August 12, 1969

Structures of pulchellin B, C, E, and F, sesquiterpene lactone constituents of a Western race of *Gaillardia pulchella* Foug., which were previously thought to be pseudoguaianolides, have been revised. Correlation of pulchellin C with derivatives of ivalin established its structure as 2α,3β-dihydroxyisolantolactone. Pulchellin B is therefore 2α-acetoxy-3β-hydroxyisolantolactone, pulchellin E is 2α-hydroxy-3β-acetoxyisoalantolactone, and pulchellin F is 2α-angeloxy-3β-hydroxyisoalantolactone. The results demonstrate that an apparent difference in H-7–H-13 couplings between C-8 *cis*-lactonized eudesmanolides on the one hand and guaianolides and pseudoguaianolides on the other can be diagnostically useful.

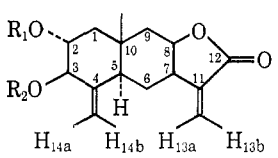
The main sesquiterpene lactone found in coastal races of *Gaillardia pulchella* Foug. is the pseudoguaianolide pulchellin (**1**).[4] Extractions of a Western race of *G. pulchella* furnished[5,6] a group of different lactones which were named pulchellin B, C, D, E, and F. Pulchellin B, C, E, and F were interrelated[5,6] and assigned pseudoguaianolide formulas **2a–d** on the basis of work with limited quantities of material. In the present communication we show that the structures of pulchellin B, C, E, and F must be revised to **3a–d**.



1



2a, R_1 = H; R_2 = Ac
b, R_1 = R_2 = H
c, R_1 = Ac; R_2 = H
d, R_1 = H; R_2 = Angeloyl



3a, R_1 = Ac; R_2 = H
b, R_1 = R_2 = H
c, R_1 = H; R_2 = Ac
d, R_1 = Angeloyl; R_2 = H
e, R_1 = R_2 = $(CH_3)_3Si$

Doubts about the structures previously assigned to pulchellin B–F resulted initially from a comparison of their nmr spectra with those of a large number of other sesquiterpene lactones that had accumulated in our laboratories. It was noted that small couplings (on the order of 1–1.5 Hz) between the H-13 and H-7 protons were characteristic of C-8 *cis*-lactonized eudesmanolide structures and that somewhat larger couplings (2–3 Hz, generally 2.5–3 Hz) between H-13 and H-7 were characteristic of guaianolides, pseudoguaianolides, and C-6 *trans*-lactonized eudesmanolides. The magnitude of the observed H-7–H-13 couplings in pulchellin B–F (1 Hz) was typical of the couplings found in C-8 *cis*-lactonized eudesmanolides and suggested a possible need for revision of previous conclusions. Indeed the data of ref 5 could be interpreted on the basis of formulas **3a** for pulchellin B and **3b** for pulchellin C if, as will be shown subsequently, certain considerations are taken into account.

In the nmr spectrum of pulchellin B, the signals of H-2 and H-3 are well separated. If it possesses formula **3a**, H-3 should be allylically coupled to the protons of the unconjugated methyl group to explain its appearance as a broad doublet. Unfortunately, the results of spin-decoupling experiments with the small amount of pulchellin B still available were equivocal. However, isolation of considerable quantities of pulchellin C from a large-scale extraction of Western *G. pulchella*[6] permitted a reinvestigation of the problem.

Examination of the 100-MHz spectrum of pulchellin C ditrimethylsilyl ether (**3e**)[7] ruled out formula **2b** for pulchellin C, since H-14a and H-14b were coupled to one of the two hydrogens under the two trimethylsilyloxy groups. Irradiation at the frequencies of H-14a or H-14b produced a positive response on the broadened doublet at 3.78 ppm; simultaneous irradiation at the frequencies of H-14a and H-14b altered the broadened doublet into a sharp doublet.[8] Conversely, irradiation at the frequency of the broadened doublet narrowed the signals of H-14a and H-14b, which in turn were slightly coupled to each other. Other observations were as follows. The multiplet at 3.5 ppm (H-2 of **3e**) was coupled to high-field signals at 1.3–1.8 ppm, suggestion that the proton responsible sor this signal was attached to a carbon atom adjacent to a methylene group. The multiplet at 3.0 ppm (H-7) was coupled to H-13a and H-13b as well as to H-8 and to signals near 1.5 ppm (C-6 methylene). Irradiation at 1.5 ppm affected the

(1) Constituents of *Gaillardia* Species. IX. Previous paper: M. Yanagita, S. Inayama, T. Kawama, T. Okura, and W. Herz, *Tetrahedron Lett.*, 2073 (1969), and errata, in press.

(2) Work at Florida State University was supported in part by a grant from the U. S. Public Health Service (GM-12408). Work at the University of Texas was supported in part by a grant from the Robert A. Welch Foundation (F-130) and the National Science Foundation (GB 5548X).

(3) To whom correspondence should be addressed.

(4) (a) W. Herz, K. Ueda, and S. Inayama, *Tetrahedron*, **19**, 483 (1963); (b) K. Aota, C. N. Caughlan, M. T. Emerson, W. Herz, S. Inayama, and Mazhar-ul-Haque, *J. Org. Chem.*, in press.

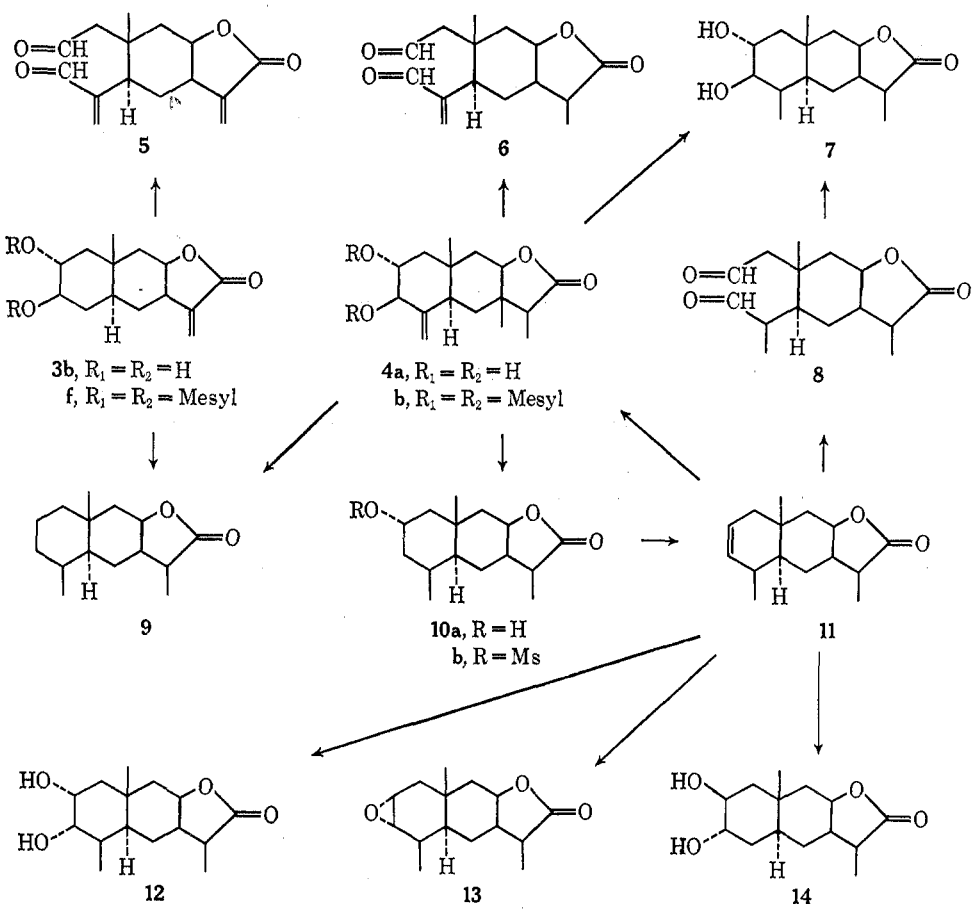
(5) W. Herz and S. Inayama, *ibid.*, **20**, 341 (1964).

(6) W. Herz and S. K. Roy, *Phytochemistry*, **6**, 661 (1969).

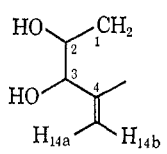
(7) In contrast to pulchellin C, this substance is freely soluble in deuteriochloroform and displays well-resolved signals at low field: 6.18 (d, 1, H-13b), 5.60 (d, 1, H-13a), 5.25 (br, H-14a), 4.71 (br, H-14b), 4.52 (br t, 5, H-8), 3.78 (br d, 9, A of AB, H-3), 3.5 (m, B of AB, H-2), 3.0 (m, H-7), 2.28 (dd, 16, 2, H-5 or H-6), and 0.82 ppm (CH_3).

(8) The high-field region at 1.3–2.4 ppm remained unaffected, indicating that H-14a and H-14b were not allylically coupled to methylene or methine protons.

SCHEME I



multiplet at 2.28 ppm (H-5 or H-6 of **3e**) as well as the signal of H-7.



A

Positive evidence for partial formula A deduced in this manner was obtained as follows (Scheme I). Periodic acid cleavage of pulchellin C and dihydropulchellin C (**4a**) resulted in the dialdehydes **5** and **6**. In these compounds the previously unconjugated exocyclic methylene group of **3b** and **4b** had become conjugated with one of the aldehyde functions, as evidenced by the downfield shift of the H-14 signals to 6.46 and 6.38 ppm in **5** and to 6.55 and 6.45 ppm in **6** as well as by the uv spectrum of **6**, λ_{max} 220 nm (ϵ 7040). Periodic acid cleavage of tetrahydropulchellin C (**7**) afforded a dialdehyde **8**, which was identical with a substance produced by ozonolysis of anhydrotetrahydroivalin (**11**) of known structure and stereochemistry.[9] This established the gross structure of pulchellin C as **3b** and the stereochemistry of ring B.

Confirmation was provided by catalytic hydrogenation–hydrogenolysis of **3f**, which resulted in the formation of tetrahydroalantolactone (**9**). Hydrogenation of the dimesylate **4b** afforded a mixture of **9** and the mesylate of tetrahydroivalin (**10b**). These unexpected results can be ascribed to initial hydrogenolysis of the allylic C-3 mesylate function. Subsequent reduction of the exocyclic double bond produces **10b**, and isomerization to a Δ^3 isomer, hydrogenolysis of the now allylic C-2 mesylate function, and further reduction produces **9**. Because of the correlation with tetrahydroivalin, the C-2 hydroxyl group of pulchellin C must be α and equatorial.

Failure of pulchellin C and its congeners to form acetonides and benzylidene derivatives suggested that the vicinal diol system was *trans*, and, because of the established configuration at C-2, $2\alpha,3.\beta$ To confirm this we studied the hydroxylation of anhydrotetrahydroivalin (**11**).

Osmylation afforded a *cis*-diol not identical with tetrahydropulchellin C. Because attack on ring A from the α side is preferred in eudesmanolides of the ivalin type as it is in steroids,[10] this substance could be assigned the $2\alpha,3\alpha$-diol formula **12**. By exclusion, therefore, tetrahydropulchellin C had to be the $2\alpha,3\beta$-diol **7**.

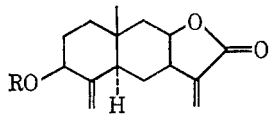
The assignment was supported by the following nmr data. (1) After the interactions between H-3 and H-14a and H-14b had been decoupled in pulchellin C, $J_{2,3}$ was found to be 9 Hz, which requires that H-3 be axial. (2) The H-14a signal occurs at 5.50 ppm in **3b**, but has experienced an upfield shift to 4.97 ppm in the 3-acetoxy derivative **3c**. A similar diamagnetic

(9) W. Herz and G. Högenauer, *J. Org. Chem.*, **27**, 905 (1962).

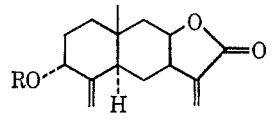
(10) See, for example, the exclusive formation of $2\alpha,3\alpha$-cholestanediol on treatment of Δ^2-cholestene with osmium tetroxide.[11]

(11) L. Fieser and M. Fieser, "Steroids," Reinhold Publishing Co., New York, N. Y., 1959, pp 274–275.

shift was observed on acetylation of 3-epiisotelekin (**15a**), which is known to have a 3β-hydroxyl group.[12] By contrast, acetylation of isotelekin (**16a**),[13] which has a 3α-hydroxyl group, results in a paramagnetic shift (from 4.95 to 5.17 ppm) of the H-14a proton. (3) In the nmr spectrum of **7** (90 MHz),[14] H-2 and H-3 appeared as an AB system in which A (H-2, 3.77 ppm) was coupled to two and B (H-3, 3.47 ppm) was coupled to one additional proton. The coupling constants, established by double irradiation, were typical of a 2,3-diequatorial alcohol [$J_{1\alpha,2}$ = 11.2 Hz (axial–axial coupling), $J_{1\beta,2}$ = 4.3 Hz (equatorial–axial), $J_{2,3}$ = 9.6 Hz (axial–axial), and $J_{3,4\alpha}$ = 5.6 Hz (axial–equatorial)].



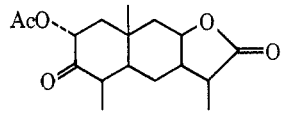
15a, R = H
b, R = Ac



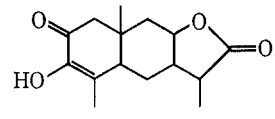
16a, R = H
b, R = Ac

Oxidation of **11** with performic acid followed by hydrolysis resulted in a single *trans*-diol, which was again not identical with tetrahydropulchellin C. The same diol was also prepared by treatment of the epoxide **13**, obtained in excellent yield from **11** with *m*-chloroperbenzoic acid (stereochemistry based on assumption of predominant attack from the α side), with perchloric acid. *trans*-Diaxial ring opening of a 2,3 epoxide (whether α or β) results in formation of a 2β,3α-diol; hence the new diol, isomeric with tetrahydropulchellin C, had to be formulated as **14**. This was consonant with the observed[14] coupling constants ($J_{2,3}$ = 3.5 Hz, $J_{3,4\alpha}$ = 2 Hz).

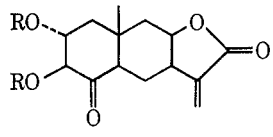
In conclusion we briefly comment on reactions previously[5] adduced in support of formula **2b** for pulchellin C. The formation of small amounts of azulenes on dehydrogenation of the mixture produced by lithium aluminum hydride reduction of **3a** and **3b** as a consequence of carbonium-ion rearrangements need occasion no surprise. The infrared spectrum of V[15] (bands at 1770, 1740, and 1725 cm^{-1}) from pulchellin B, which was attributed to the presence of a cyclopentanone ring, is equally explicable in terms of structure **15** (equatorial α-acetoxy ketone) derived from **3a**. The diosphenol X produced by oxidation of tetrahydropulchellin C now becomes **16** (vinyl methyl, but no vinyl proton signal in nmr spectrum). The misleading positive Zimmerman test of the apo ketone XII which requires reformulation as **17b** may plausibly be ascribed to deacetylation followed by dehydration under the strongly alkaline condition of the test to a compound **18a** whose formula is compatible with the properties of a diosphenol previously formulated as XV.[16] That XIV is the acetate **18b**, the empirical formula of which also satisfies the analytical figures, could be shown by repetition of the experiment. The product, identical with the material isolated earlier, displayed the expected nmr signals (acetate singlet at 2.26 ppm, vinyl triplet at 6.59 ppm). Hydrolysis of **18b** gave **18a**, whose properties were indeed identical with the properties of the substance previously[5] formulated as XV.



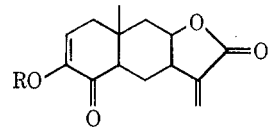
15



16



17a, R = H
b, R = Ac



18a, R = H
b, R = Ac

Experimental Section[17]

Pulchellin C Ditrimethylsilyl Ether (3e).—To a solution of 80 mg of pulchellin C in 0.5 ml of anhydrous pyridine was added 0.5 ml of trimethylchlorosilane and 0.5 ml of hexamethyldisilazane. After 5 min the mixture was evaporated to dryness at reduced pressure. The residue was extracted with anhydrous carbon tetrachloride, filtered in a dry atmosphere, and washed with a few milliliters of carbon tetrachloride. The filtrate and washings were evaporated *in vacuo*. Recrystallization of the residual crude trimethylsilyl ether from the minimum amount of cyclohexane afforded 50 mg of prisms, mp 157–158°, which contained 1 mol of cyclohexane as a solvate. The solvent of crystallization could be removed by dissolving the solvate in absolute chloroform and evaporating the solution to dryness. The product had ir bands at 1750, 1645, 1262, 1250, and 840 cm^{-1}. The spin-decoupling experiments were performed on a Varian 100-MHz instrument in deuteriochloroform solution.

Dimesylpulchellin C (3f).—A solution of 200 mg of pulchellin C in 0.5 ml of dry pyridine was mixed with a solution of 1 ml of mesyl chloride in 0.5 ml of dry pyridine under cooling. After 7 hr at room temperature, 5 ml of chloroform was added. The solution was washed with water and dried, and the chloroform layer was concentrated *in vacuo*. The crude residue, yield 0.3 g, was recrystallized from ethyl acetate, yield of first crop 175 mg, yield of second crop 35 mg. Further recrystallization from ethyl acetate afforded the analytical sample of **3f**: mp 177–178° dec; ir (Nujol) 1755, 1170, and 840 cm^{-1}; nmr ($CDCl_3$) 6.22 (d) and 5.71 (d) (1, H-13a and H-13b), 5.50 (m, $W_{1/2}$ = 4 Hz, H-14a), 5.0 (m, $W_{1/2}$ = 4 Hz, H-14b) superimposed on 5 (c, H-3), 4.6 (c, 2, H-2 and H-8), 3.18 and 3.12 (mesylates), 3.0 (c, H-7), and 0.92 ppm (C-10 methyl).

Anal. Calcd for $C_{17}H_{24}O_8S_2$: C, 48.55; H, 5.72; O, 30.50; S, 15.23. Found: C, 48.39; H, 5.68; O, 30.58; S, 15.22.

Hydrogenolysis of Dimesylpulchellin C.—A sample of 45 mg of **3f**, twice recrystallized from ethyl acetate,[18] was hydrogenated in the presence of 45 mg of platinum oxide which had been prehydrogenated in 15 ml of glacial acetic acid. After 20 hr, 13 ml of hydrogen (*ca.* 5 molar equiv) had been absorbed. The solution was filtered and evaporated *in vacuo;* the residue was dissolved in 3 ml of chloroform, washed, dried, and evaporated. Preparative tlc of the crystalline residue over silica gel G ($CHCl_3$, R_f 0.67) followed by recrystallization from petroleum ether yielded 10 mg of tetrahydroalantolactone (**9**), mp 136–138°, $[\alpha]_D$ +8° (*c* 1, $CHCl_3$), identical (ir, nmr) in all respects with an authentic sample.

When once-recrystallized **3f** was used, the catalyst became poisoned before hydrogenolysis occurred. Thus 300 mg of **3f**, recrystallized once from ethyl acetate, on hydrogenation with 100 mg of prereduced platinum oxide in glacial acetic acid afforded

(12) W. Herz, P. S. Subramaniam, and T. A. Geissman, *J. Org. Chem.*, **33**, 3743 (1968).

(13) V. Benesova, V. Herout, and F. Sorm, *Collect. Czech. Chem. Commun.*, **26**, 1350 (1961).

(14) Determined in deuteriochloroform on a 90-MHz Bruker nmr spectrometer purchased with the aid of a grant from the National Science Foundation. We are indebted to Mr. A.L .Hall for carrying out the decoupling experiments.

(15) Roman numerals refer to the structures given in ref 5.

(16) This substance was obtained only in small amounts and an nmr spectrum was not available. An attempt to repeat its preparation by the method given in ref 5 failed.

(17) The experimental conditions given in ref 4b apply, unless otherwise specified. Pulchellin C was isolated as described in ref 6.

(18) Two recrystallizations of **3f** from ethyl acetate were necessary to effect hydrogenolysis of **3f** to **9** (*vide infra*).

after 16 hr (32 ml hydrogen uptake) 0.3 g of solid residue. Recrystallization from chloroform–ethyl acetate yielded 253 mg of dimesyldihydropulchellin C (**4b**): mp 173° dec; ir (Nujol) 1748, 1640, 1175, 850, 843, and 835 cm^{-1}; nmr (DMSO) 5.32 (m, $W_{1/2}$ = 4 Hz, H-14a), 5.0 (m, $W_{1/2}$ = 4 Hz, H-14b) partially superimposed on 5.1 (c, H-3), 4.5 (c, 2, H-2 and H-8), 3.22 (two mesylates), 1.10 (d, 7, C-11 methyl), and 0.76 ppm (C-10 methyl).

Anal. Calcd for $C_{17}H_{26}O_8S_2$: C, 48.35; H, 6.17; O, 30.35; S, 15.18. Found: C, 48.16; H, 6.31; O, 30.14; S, 15.07.

Hydrogenolysis of 4b.—Dimesyldihydropulchellin (**4b**), wt 182 mg, was hydrogenated with 60 mg of prereduced platinum oxide in glacial acetic acid. After 17 hr (30-ml hydrogen uptake, *ca.* 3 molar equiv), the reaction mixture was worked up in the usual way. Preparative tlc of the gummy product over silica gel G with chloroform yielded two major components. A band with R_f 0.60 gave 50 mg of solid material which, after recrystallization from petroleum ether, melted at 146–147°, $[\alpha]D$ 48.1° (*c* 3.2), and was identical with authentic tetrahydroalantolactone, mp 147–148°, $[\alpha]D$ +11° (*c* 3), by mixture melting point and ir and nmr spectrum. A second band with R_f 0.13 afforded 83 mg of solid which was recrystallized from 1:1 ethyl acetate–cyclohexane: mp 138–139°; $[\alpha]D$ +7.7° (*c* 2.8); identical in all respects (mixture melting point, ir and nmr spectrum) with a sample of O-mesyltetrahydroivalin, $[\alpha]D$ +8.9° (*c* 3.3).[9]

Periodic Acid Oxidation of Pulchellin C.—To a solution of 0.35 g of **3b** in 10 ml of tetrahydrofuran was added an excess of a solution of periodic acid in tetrahydrofuran. A precipitate of iodic acid formed immediately. After 5 min the solution was poured into water and extracted with ether. Removal of solvent from the dried ether layer afforded 0.33 g of crude **5**, which was recrystallized from hexane–tetrahydrofuran: mp 108–110°; $[\alpha]D$ +37° (*c* 1.4, absolute ethanol); λ_{max} 213 nm (ϵ 9400); ir 1770, 1725, 1695, and 1615 cm^{-1}; nmr 9.94 (t, 2.5, H-2), 9.68 (H-3), 6.46 (br) and 6.38 (br) (H-14), 6.25 (d, 1) and 5.70 (d, 1, H-13), 4.66 (c, H-7), and 1.11 ppm (C-10 methyl).

Anal. Calcd for $C_{15}H_{18}O_4$: C, 68.68; H, 6.92; O, 24.40. Found: C, 68.68; H, 7.12; O, 24.14.

Periodic Acid Oxidation of Dihydropulchellin C.—Oxidation of 0.5 g of **4a** in the manner described in the previous section yielded 0.48 g of crude **6**, which was recrystallized from hexane–tetrahydrofuran: mp 128–130°; $[\alpha]D$ −34.1° (*c* 1.23, ethanol); λ_{max} 220 nm (ϵ 7040); ir 1765, 1715, 1685, and 1610 cm^{-1}; nmr 9.96 (t, 2.5, H-2), 9.71 (H-3), 6.55 (br) and 6.45 (br) (H-14), 4.64 (c, H-8), 3.03 (q, 6, H-11), 1.21 (d, 7, C-11 methyl), and 1.11 ppm (C-10 methyl).

Anal. Calcd for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63; O, 24.21. Found: C, 67.92; H, 7.67; O, 24.31.

Formation of 8. A.—Periodic acid oxidation of 0.8 g of tetrahydropulchellin C in the usual manner afforded 0.79 g of a gum (**8**) which could not be induced to crystallize. It had nmr signals at 9.91 (t, 2.5, H-2), 9.81 (d, 2.5, H-3), 4.48 (c, H-8), 2.76 (q, 6, H-11), 1.17 (d, 7, C-11 methyl), 1.14 (d, 7, C-4 methyl), and 1.15 ppm (C-10 methyl).

B.—A solution of 0.28 g of 11[9] in 20 ml of methanol was ozonized at 0° until the blue color of ozone was permanent. The solution was hydrogenated over 0.05 g of 5% Pd–$CaCO_3$ at atmospheric pressure, filtered, and evaporated. Chromatography over Florisil gave a product which was identical with **8** in all respects (nmr, ir, tlc).

Preparation of 12.—A solution of 0.51 g of 11 in 15 ml of anhydrous pyridine was mixed with 0.56 g of osmium tetroxide and allowed to stand for 2 hr. A black precipitate formed and was decomposed with a solution of 1.8 g of sodium metabisulfite in 60 ml of 1:1 water–pyridine. The mixture was stirred for 30 min and extracted with methylene chloride. The washed and dried extract was evaporated and the residual oil, wt 0.43 g, was chromatographed over Florisil. Elution with ethyl acetate gave a fraction which, after recrystallization from ethyl acetate–heptane, gave 0.34 g of **12**: mp 138–139°; $[\alpha]D$ +26.6° (*c* 1.5, ethanol); ir 3560, 3460, and 1760 cm^{-1}.

Anal. Calcd for $C_{15}H_{24}O_4$: C, 67.14; H, 9.01; O, 23.85. Found: C, 67.14; H, 9.06; O, 24.56.

Epoxidation of Anhydrotetrahydroivalin.—A solution of 0.45 g of 11 in 15 ml of chloroform was refluxed with excess *m*-chloroperbenzoic acid for 4 hr. The solution was cooled, poured into ice-cold water, and extracted into ether. The organic layer was washed, dried, and evaporated, and the residue, wt 0.46 g, was purified by chromatography over alumina. Recrystallization from ethyl acetate–hexane gave 0.37 g of **13**: mp 175°; $[\alpha]D$ +20.8° (*c* 0.55, ethanol); ir 1760 cm^{-1}; nmr 4.50 (c, H-8), 3.1 (c, H-2 and H-3), 2.78 (q, 6, H-1), 1.22 (d, 7, C-11 methyl), 1.06 (d, 8, C-4 methyl), and 0.98 ppm (C-10 methyl).

Anal. Calcd for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86; O, 19.17. Found: C, 72.02; H, 8.58; O, 19.59.

Preparation of 14. A.—A mixture of 0.35 g of 11 and 2.5 ml of 30% hydrogen peroxide solution in 15 ml of 90% formic acid was allowed to stand at room temperature for 1 hr and then warmed on the steam bath for 1 hour. After the addition of 10 ml of potassium carbonate solution (1:1 water–methanol), the mixture was allowed to stand overnight and then extracted into ether. The washed and dried extract was evaporated at reduced pressure and the gummy residue, wt 0.32 g, was chromatographed over basic alumina. Elution with 19:1 ether–methanol gave crystalline **14**, which was recrystallized from ethyl acetate–hexane: mp 205–206°; $[\alpha]D$ +20.6° (*c* 0.68, ethanol); ir 3580, 3460, and 1760 cm^{-1}; nmr (acetone-d_6) 4.44 (m, H-8), 4.15 (m, H-2), 3.65 (m, H-3, $J_{2,3}$ = 3.5 Hz, $J_{3,4}$ = 2 Hz), 2.80 (quintet, H-11), 1.11 (C-10 methyl), and 1.06 (d, 7.2) and 1.02 (d) ppm (7.7, C-4 and C-11 methyl).

Anal. Calcd for $C_{15}H_{24}O_4$: C, 67.14; H, 9.01; O, 23.85. Found: C, 67.09; H, 8.99; O, 23.97.

B.—A solution of 0.096 g of **13** in 5 ml of acetone was allowed to stand for 2 hr with 10 ml of a 7% solution of perchloric acid in acetone, poured into ice–water, and extracted into ether. The organic layer was washed, dried, and evaporated and the residual gum was chromatographed over alumina. Elution with 9:1 ether–methanol gave **14**, mp 205–206°, which was identical in all respects with the material prepared as described in the previous paragraph.

Preparation of 18b.—A solution of 0.46 g of diacetylapodihydropulchellone C (**17b**)[5] in 10 ml of pyridine was refluxed for 12 hr, cooled, poured into ice–water, and extracted into ether. The organic layer was washed, dried, and evaporated. The solid residue, wt 0.44 g, was recrystallized from acetone–hexane to yield 0.39 g of **18b**: mp 230°; $[\alpha]D$ +12.8° (*c* 0.39, $CHCl_3$); λ_{max} 234 nm (ϵ 8860); ir 1760 and 1690 cm^{-1}; nmr 6.59 (t, 4.5, H-2), 4.6 (c, H-8), 2.86 (q, 6, H-11), 2.26 (acetate), 1.26 (d, 7, C-11 methyl), and 1.08 ppm (C-10 methyl).

Anal. Calcd for $C_{16}H_{20}O_5$: C, 65.74; H, 6.90; O, 27.36. Found: C, 65.69; H, 6.93; O, 27.53.

The previously unreported nmr spectrum of **17a** (dimethyl sulfoxide-d_6) exhibited signals at 4.86 (c, H-2), 4.70 (c, H-3), 4.61 (c, H-8), 2.95 (q, 6, H-11), 1.23 (d, C-11 methyl), and 0.93 ppm (C-10 methyl).

Treatment of **17a** with acetic anhydride–pyridine and decomposition with warm water did not result in dehydration to **18a**, but gave **17b**. Corrected values for the nmr spectrum of **17b** are 5.35 (c, H-2), 5.30 (c, H-3), 4.56 (c, H-8), 2.85 (q, 6, H-11), 2.18 and 2.08 (acetates), 1.23 (d, 7, C-11 methyl), and 0.95 ppm (C-10 methyl). However, conversion of **18b** into **18a** was effected as follows. A solution of 0.07 g of **18b** in 5 ml of methanol was refluxed overnight with 5 ml of a 5% solution of hydrochloric acid in methanol. The mixture was poured into water and extracted into ether. The organic layer was washed, dried, and evaporated and the residual gum was recrystallized from aqueous methanol to give pale yellow crystals, mp 128–130°, identical with the material previously formulated as XV by direct comparison. The nmr spectrum exhibited signals at 6.21 (t, 4.5, H-2), 4.7 (c, H-8), 1.36 (C-10 methyl), and 1.32 (d, 7, C-11 methyl).

The nmr spectrum of bisdehydrotetrahydropulchellin C (**16**) exhibited signals at 4.5 (c, H-8), 1.88 (d, 1.5, C-4 methyl), 1.24 (d, 7, C-11 methyl), and 0.99 ppm (C-10 methyl).

The nmr spectrum of **7** (90 MHz)[14] exhibited the following signals: 4.42 (m, H-8), 3.77 (m, A of AB, H-2), 3.47 (q, B of AB, H-3), 2.78 (quintet, H-11), 2.07 (dd, H-9β), 1.87 (dd, H-1β), 1.19 (C-11 methyl, superimposed on H-1α), 1.02 (d, C-10 methyl), and 0.82 (d, C-4 methyl). The coupling constants (Hz) were H-1α,H-1β = −13; H-1α,H-2 = 11.2; H-1β,H-2 = 4.3; H-2,H-3 = 9.6; H-3,H-4 = 5.6; H-4,C-11 Me = 7.2; H-7,H-8 = 4; H-7,H-11 = 6; H-8,H-9α = 4; H-8,H-9β = 2; H-9α,H-9β = −14.6; H-11,C-11 Me = 7.

Acetylisotelekin (16b).—Isotelekin (**16a**) was acetylated under the same conditions used for epiisotelekin (**15a**).[12] The product was recrystallized from cyclohexane–benzene: mp 128°; nmr 6.17 (d, 1, H-13a), 5.63 (d, 1, H-13b), 5.4 (m, $W_{1/2}$ = 6 Hz, H-3), 5.17 (c, $W_{1/2}$ = 3.5 Hz, H-14a), 4.75 (c, $W_{1/2}$ = 3.5,H-14b), 4.56 (td, 5.5, 2, H-8), 3.06 (H-7), 2.07 (acetate), and 0.85 ppm (C-10 methyl).

Registry No.—**3a**, 22850-58-4; **3b**, 22850-59-5; **3c**, 22850-60-8; **3d**, 22850-61-9; **3f**, 22850-62-0; **4b**, 22850-63-1; **5**, 22850-64-2; **6**, 22850-65-3; **7**, 22850-66-4; **12**, 22850-67-5; **13**, 22850-68-6; **14**, 22922-41-4; **16**, 22850-69-7; **16b**, 22850-70-0; **17a**, 24375-89-1; **17b**, 22850-71-1; **18b**, 22850-72-2.

Purine Nucleosides. XXVI. A General Synthesis of 6-Substituted 7-(β-D-Ribofuranosyl)purines. A Reinvestigation and Corroboration of the Position of Glycosylation of 6-Dimethylamino-"7"-(β-D-ribofuranosyl)purine[1]

ROBERT J. ROUSSEAU,[2a] RAYMOND P. PANZICA,[2b] STEPHEN M. REDDICK, ROLAND K. ROBINS, AND LEROY B. TOWNSEND[3]

Department of Chemistry and Department of Biopharmaceutical Sciences, University of Utah, Salt Lake City, Utah 84112

Received July 22, 1969

A general method for the synthesis of 6-substituted 7-(β-D-ribofuranosyl)purines has been achieved *via* ring closure of an imidazole nucleoside. The preparation of 7-(β-D-ribofuranosyl)purine-6-thione (**7**) from 4-amino-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-5-carboxamide (**1**) has provided a route for the production of 6-alkylthio-, 6-alkylamino-, and 6-alkoxy-7-(β-D-ribofuranosyl)purines. An unambiguous synthesis of 6-dimethylamino-7-(β-D-ribofuranosyl)purine (**14**) has verified a previous investigation which shows that the site of glycosylation reported in the literature for 6-dimethylamino-"7"-(β-D-ribofuranosyl)purine was in error. The glycosyl linkage of 6-ethoxy-7-(β-D-ribofuranosyl)purine (**5**) has been shown to be unusually labile toward dilute sodium ethoxide solutions which generally do not affect purine nucleoside glycosidic bonds.

Considerable interest in the synthesis of 7-glycosylpurines was generated when the purine nucleoside isolated from pseudovitamin B_{12} was characterized as 7-α-D-ribofuranosyladenine. Other nucleosides which were isolated from pseudovitamin B_{12} analogs were also assumed to be 7-ribosylpurines.[4] Many of the nucleosides reported[5-9] in the literature which have been assigned as 7-glycosylpurines have been isolated only as minor products from a mixture by lengthy separation procedures. The preparation of 7-ribosylpurines *via* direct glycosylation of a preformed purine has also been shown to suffer from several inherent difficulties.[10,11] Recently, the synthesis of 6-substituted 7-glycosylpurines has been achieved from imidazole nucleosides,[12] but the routes[13-15] used were restrictive in that they provided only an amino or keto group at position 6 of the purine ring for monosubstituted nucleosides. It has been suggested that the structural assignments for several previously reported 6-substituted 7-glycosylpurines are questionable[16] and, therefore, a general method for the *unambiguous* synthesis of these nucleosides seemed desirable. This prompted the present investigation for a 6-substituted 7-(β-D-ribofuranosyl)-purine with a functional group at position 6 which would be amenable toward nucleophilic displacement. The ring closure of an imidazole nucleoside to a 7-glycosylpurine with a methylthio group at position 6 has been accomplished and was followed by the appropriate functional group transformations to achieve that goal.

An attempt was made to synthesize 7-(β-D-ribofuranosyl)purine-6-thione (**7**) from 4-amino-5-cyano-1-(β-D-ribofuranosyl)imidazole (**2**) by the usual procedure (treatment with a mixture of ethyl orthoformate–acetic anhydride, followed by ethanolic sodium hydrogen sulfide).[17] The crystalline solid isolated from this reaction mixture exhibited two spots by paper chromatography. Several recrystallizations from water gave a very small yield of a product with physical properties which were incompatible with the expected structure **7**. This observation was based partly on the pmr spectrum of the solid in dimethyl sulfoxide-d_6 which exhibited only one singlet (δ 8.90, one proton) in the region where the H_2 and H_8 signals were expected (δ 8 ± 1) as well as an unexpected signal at δ 2.5 (three protons). On the basis of elemental analysis and pmr spectra, the nucleoside was assigned the structure 2-methyl-7-(β-D-ribofuranosyl)purine-6-thione (**4**). Formation of **4** can be rationalized by the formation of the 4-N-acetyl intermediate **3** presumably *via* the facile reaction of excess acetic anhydride with the 4-amino group of 4-amino-5-cyano-1-(β-D-ribofuranosyl)imidazole (**2**, Scheme I), followed by annulation.

From the pmr spectrum in D_2O of the initial crystalline mixture it was calculated that **4** and another compound which was subsequently shown to be the desired

(1) This work was supported by Research Contract No. PH-43-65-1041 with Chemotherapy, National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service.

(2) (a) This manuscript was part of a Ph.D. Thesis by R. J. Rousseau, University of Utah, 1969. (b) The recipient of a University of Utah Research Committee Fellowship, 1968–1969.

(3) Author to whom inquiries may be addressed at the University of Utah.

(4) For a recent and comprehensive review of vitamin B_{12} and naturally occurring analogs of vitamin B_{12}, the reader is referred to R. Bonnett, *Chem. Rev.*, **63**, 573 (1963); E. L. Smith, "Vitamin B_{12}," 2nd ed, Methuen Co., London, 1963; K. Bernhauer, O. Muller, and F. Wagner, *Angew. Chem. Intern. Ed. Engl.*, **3**, 200 (1964).

(5) H. M. Kissman, C. Pidacks, and B. R. Baker, *J. Amer. Chem. Soc.*, **77**, 18 (1955).

(6) Y. Misuno, M. Ikehara, T. Itoh, and K. Sato, *J. Org. Chem.*, **30**, 4066 (1965).

(7) J. Baddiley, J. G. Buchanan, G. O. Osbourne, *J. Chem. Soc.*, 3606 (1958).

(8) K. Amai, A. Nohara, and M. Honjo, *Chem. Pharm. Bull.* (Tokyo), **14**, 1377 (1966).

(9) T. Hashizume and H. Iwamura, *J. Org. Chem.*, **33**, 1796 (1968).

(10) J. A. Montgomery and H. J. Thomas, *ibid.*, **28**, 2304 (1963).

(11) H. J. Thomas and J. A. Montgomery, *ibid.*, **31**, 1413 (1966).

(12) L. B. Townsend, *Chem. Rev.*, **67**, 533 (1967).

(13) R. J. Rousseau, L. B. Townsend, and R. K. Robins, *Chem. Commun.*, 265 (1966).

(14) R. J. Rousseau, R. K. Robins, and L. B. Townsend, *J. Amer. Chem. Soc.*, **90**, 2661 (1968).

(15) R. J. Rousseau and L. B. Townsend, *J. Org. Chem.*, **33**, 2828 (1968).

(16) L. B. Townsend, R. K. Robins, R. N. Loeppky, and N. J. Leonard, *J. Amer. Chem. Soc.*, **86**, 5320 (1964).

(17) E. C. Taylor, A. McKillop, and R. N. Warrener, *Tetrahedron*, **23**, 885 (1967).