# Structure of Lansiosides: Biologically Active New Triterpene Glycosides from *Lansium domesticum*[1]

Mugio Nishizawa,*† Hisaya Nishide,† Soleh Kosela,‡ and Yuji Hayashi†

*Departments of Chemistry, Faculty of Science, Osaka City University, Osaka 558, Japan, and University of Indonesia, Jakarta, Indonesia*

*Received May 9, 1983*

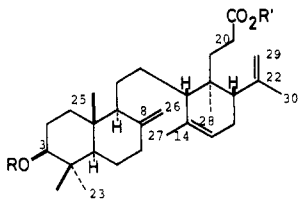
From the extract of the fruit peel of *Lansium domesticum* (Meliaceae), three new triterpene glycosides, named lansiosides A, B, and C, have been isolated. Their structures and absolute configurations have been established to be **1**, **2**, and **3**, respectively, by spectral analysis and chemical derivatization. Each lansioside provides on methanolysis the same aglycon, methyl lansiolate (**4**), which is a representative of a class of new triterpenoids with a secoonocerane framework. Lansioside A (**1**) is a very rare example of an amino sugar glycoside from a plant and has unique biological behavior. Lansioside A inhibits the leukotriene $D_4$ induced contraction of guinea pig ileum in 2.4 ppm concentration effectively.

The fruit of *Lansium domesticum* Jack var. Duku (Meliaceae) is a very popular dessert of the local name duku in Indonesia. However, the peel of the fruits has been traditionally said to be toxic to domestic animals. We have investigated the biologically active constituents of this fruit peel, easily available anywhere in Indonesia during the rainy season (January to April), and have isolated three novel glycosides.[2] The peel contains a large quantity of latex. The major component of the latex has already been shown to be lansic acid (**5**) through the chemical correlation with $\alpha$-onocerin (**6**).[3] Herein described are the isolation and structure determination of three glycosides, lansiosides A, B, and C, of a new type of triterpene with the secoonocerane skeleton, obtained from more polar fractions than lansic acid. One of the glycosides, lansioside A (**1**, Chart I), contains an *N*-acetylglucosamine moiety as the sugar part, which is a novel example of an amino sugar glycoside as a plant constituent. Investigation of its biological activity has revealed that this compound shows an inhibitory activity of the leukotriene $D_4$ induced contraction of guinea pig ileum.

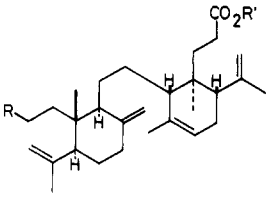
## Results and Discussion

The dried peel of *L. domesticum* (2 kg) was crushed and extracted with ethanol. Fractionation of the ethanol-soluble portion afforded lansiosides A, B, and C along with lansic acid (**5**). Lansioside A (**1**), mp 174–175 °C, $C_{38}H_{61}NO_8 \cdot H_2O$, was obtained as colorless needles in ca. 0.36% yield based on the dried materials. The IR and NMR spectra suggested the presence of aceylamino (1640 $cm^{-1}$) and carboxy moieties together with hydroxyl groups. Lansioside A (**1**) afforded methyl ester triacetate **7** by successive treatment with acetic anhydride/pyridine and diazomethane. This derivative gave well-resolved $^1H$ and $^{13}C$ NMR spectra, which indicated the structure of lansioside A as a triterpene glycoside derivative. Acid-catalyzed methanolysis of **1** gave aglycon methyl ester **4** and an anomeric mixture of methylated sugar derivatives.[4] The latter mixture was transformed into acetates and purified by silica gel column chromatography to give pure methyl 2-acetamido-2-deoxy-$\alpha$-D-glucopyranoside triacetate (**16**) and its $\beta$-anomer **17**.[5] These two methyl glycosides were totally identified by comparison with authentic samples, including optical rotation. Since lansioside A (**1**) originally possesses an acetyl moiety on the nitrogen atom, the sugar part of the glycoside was unambiguously confirmed to be *N*-acetyl-D-glucosamine, which is connected to the sole hydroxyl group (C-3) of the triterpene at the anomeric position.[6] The stereochemistry at the anomeric carbon of lansioside A was determined to be $\beta$-glycosidyl on the basis of the NMR spectra of its methyl ester triacetate **7**. Signals due to the anomeric proton and carbon appeared at $\delta$ 4.67 (d, $J$ = 8 Hz) and 103.3, respectively.[7]

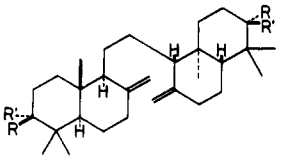
Chart I



1, R = *N*-acetyl-β-D-glucosamine ; R' = H
2, R = β-D-glucose ; R' = H
3, R = β-D-xylose ; R' = H
4, R = H ; R' = $CH_3$
7, R = *N*-acetyl-β-D-glucosamine triacetate ; R' = $CH_3$
8, R = β-D-glucose tetracetate ; R' = $CH_3$
9, R = β-D-xylose triacetate ; R' = $CH_3$
10, R = H ; R' = H



5, R = $CO_2H$ ; R' = H
11, R = $CO_2CH_3$ ; R' = $CH_3$
15, R = CN ; R' = $CH_3$



6, R = OH ; R' = H
13, R = R' = O

(1) Dedicated to Emeritus Professor Takeo Sakan of Osaka City University on the 70th anniversity of his birth.

(2) This study was partly reported as a communication, see: Nishizawa, M.; Nishide, H.; Hayashi, Y.; Kosela, S. *Tetrahedron Lett.* **1982**, *23*, 1349.

(3) Kiang, A. K.; Tan, E. L.; Lim, F. Y.; Habaguchi, K.; Nakanishi, K.; Fachan, L.; Ourisson, G. *Tetrahedron Lett.* **1967**, 3571. Habaguchi, K.; Watanabe, M.; Nakadaira, Y.; Nakanishi, K.; Kiang, A. K.; Lim, F. Y. *Ibid.* **1968**, 3731.

(4) Preliminary information for the sugar moiety was obtained by acid hydrolysis (1 N HCl/100 °C) followed by TLC analysis (Merck Art. 5721 plate, butanol, pyridine, water (8:1:1) solvent system). Identification of glucosamine was also carried out by using an amino acid analyser (Hitachi 835 instrument). We thank Professor T. Yamamoto and Dr. M. Iizuka of the same faculty for valuable advice and amino sugar analysis.

(5) Paul, B.; Bernacki, R. J.; Korytnyk, W. *Carbohydr. Res.* **1980**, *80*, 99.

(6) Bruneton, J.; Cavé, A. *Phytochemistry* **1972**, *11*, 846. Ripperger, H.; Preiss, A.; Schmidt, J. *Ibid.* **1981**, *20*, 2434.

† Osaka City University.
‡ University of Indonesia.

0022-3263/83/1948-4462$01.50/0 

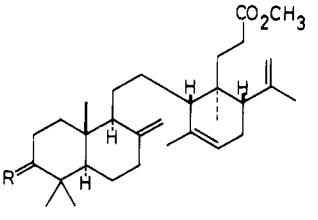
Table I. $^{13}C$ NMR Spectral Data (25 MHz, $CDCl_3$ or Pyridine-$d_5$)[g]

| | compound | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| carbon | 1 | 7 | 2 | 8 | 3 | 9 | 4 | 12 | 5 | 6 |
| 1 | 38.4[a] | 38.2[a] | 38.4[a] | 38.2[a] | 38.6[a] | 38.0[a] | 38.1[a] | 37.8[a] | 28.7[a] | 38.5[a] |
| 2 | 29.9 | 29.1 | 28.4 | 29.1 | 30.1 | 28.9 | 28.0 | 34.8 | 32.1 | 28.5 |
| 3 | 89.1 | 89.8 | 88.8 | 90.5 | 88.7 | 89.6 | 78.8 | 217.1 | 181.7 | 79.1 |
| 4 | 39.5[b] | 39.4[b] | 39.8[b] | 39.3[b] | 40.1[b] | 39.3[b] | 39.4[b] | 47.8 | 147.0[b] | 39.5[b] |
| 5 | 58.1 | 58.1 | 58.4 | 58.2 | 58.4 | 58.2 | 58.2 | 57.6 | 47.7[c] | 57.8 |
| 6 | 24.3 | 24.0 | 24.2 | 24.1 | 24.5 | 23.8 | 24.0 | 25.2 | 29.7 | 24.3 |
| 7 | 337.3[a] | 37.2[a] | 37.4[a] | 37.2[a] | 37.6[a] | 37.1[a] | 37.1[a] | 37.7[a] | 38.2 | 37.3[a] |
| 8 | 148.9 | 148.1 | 149.0 | 148.1 | 148.9 | 148.0 | 148.2 | 147.7 | 148.0 | 148.4 |
| 9 | 54.5 | 55.2 | 54.9 | 55.0 | 55.1 | 54.8 | 54.5 | 55.1 | 50.8[d] | 54.9 |
| 10 | 39.5[b] | 39.2[b] | 39.5[b] | 39.3[b] | 39.7[b] | 39.1[b] | 39.1[b] | 39.4 | 41.6 | 39.3[b] |
| 11 | 26.0[c] | 25.7[c] | 26.0[c] | 25.7[c] | 26.2[c] | 25.8[c] | 25.8[c] | 26.2[c] | 27.5[e] | 22.9 |
| 12 | 27.1[c] | 26.0[c] | 27.3[c] | 26.7[c] | 27.7[c] | 26.6[c] | 27.2[c] | 27.2[c] | 27.9[e] | |
| 13 | 48.3[d] | 48.3[d] | 48.4[d] | 48.3[d] | 48.3[d] | 49.3[d] | 48.2[d] | 48.3[d] | 51.7[d] | |
| 14 | 136.4 | 136.0 | 136.4 | 136.1 | 136.4 | 136.0 | 136.0 | 136.0 | 135.7 | |
| 15 | 122.0 | 121.8 | 122.0 | 121.8 | 122.0 | 121.7 | 121.7 | 122.0 | 122.0 | |
| 16 | 29.9 | 29.6 | 29.9 | 29.7 | 30.1 | 29.3 | 29.5 | 29.5 | 30.5 | |
| 17 | 48.9[d] | 49.3[d] | 48.9[d] | 49.3[d] | 49.1[d] | 49.1[d] | 49.1[d] | 49.2[d] | 48.9[c] | |
| 18 | 38.9[b] | 38.7[b] | 39.0[b] | 38.8[b] | 39.2[b] | 38.6[b] | 38.6[b] | 38.7 | 38.8 | |
| 19 | 27.7 | 27.2 | 27.6 | 27.2 | 27.7 | 27.2 | 28.9 | 29.0 | 29.2[a] | |
| 20 | 39.9 | 33.1 | 33.9 | 33.1 | 34.0 | 32.8 | 32.8 | 32.9 | 33.1 | |
| 21 | 176.4 | 174.5 | 176.4 | 174.6 | 176.2 | 174.4 | 174.5 | 174.7 | 181.7 | |
| 22 | 148.2 | 147.7 | 148.2 | 147.8 | 148.1 | 147.6 | 147.6 | 147.8 | 147.4[b] | |
| 23 | 28.2 | 28.2 | 28.4 | 28.1 | 28.6 | 28.0 | 28.3 | 26.1 | 23.9[f] | 28.2 |
| 24 | 16.9 | 16.4 | 17.0 | 16.3 | 17.0 | 16.2 | 15.4 | 21.7 | 113.8 | 15.7 |
| 25 | 14.8 | 14.6 | 14.9 | 14.7 | 15.1 | 14.6 | 14.6 | 14.2 | 17.9 | 14.8 |
| 26 | 107.0 | 107.0 | 107.0 | 107.1 | 107.0 | 106.9 | 106.7 | 107.8 | 107.3 | 106.8 |
| 27 | 23.3 | 23.0 | 23.3 | 23.1 | 23.5 | 22.9 | 22.9 | 22.9 | 23.3 | |
| 28 | 17.0 | 16.6 | 19.2 | 16.6 | 17.2 | 16.4 | 16.4 | 16.4 | 16.1 | |
| 29 | 114.0 | 113.9 | 114.0 | 114.0 | 114.0 | 113.8 | 113.8 | 114.7 | 114.1 | |
| 30 | 23.3 | 23.0 | 23.3 | 23.1 | 23.5 | 22.9 | 22.9 | 22.9 | 22.8[f] | |
| 1′ | 104.6 | 103.3 | 106.7 | 103.1 | 107.4 | 103.0 | | | | |
| 2′ | 58.3 | 55.0 | 75.7 | 71.9 | 75.4 | 71.5 | | | | |
| 3′ | 76.0 | 71.6 | 78.1 | 71.9 | 78.4 | 71.9 | | | | |
| 4′ | 72.5 | 69.0 | 71.7 | 68.9 | 71.2 | 69.1 | | | | |
| 5′ | 78.1 | 72.5 | 78.7 | 73.0 | 67.0 | 62.1 | | | | |
| 6′ | 62.8 | 62.5 | 62.9 | 62.3 | | | | | | |
| NAc | 23.7 | 23.0 | | | | | | | | |
| | 170.4 | 170.6 | | | | | | | | |
| OMe | | 51.5 | | 51.6 | | 51.4 | 51.5 | 51.7 | | |
| OAc | | 20.8 | | 20.8 | | 20.7 | | | | |
| | | 169.4 | | 169.1 | | 169.1 | | | | |
| | | 170.0 | | 169.4 | | 169.7 | | | | |
| | | 171.0 | | 170.4 | | 170.0 | | | | |
| | | | | 170.7 | | | | | | |

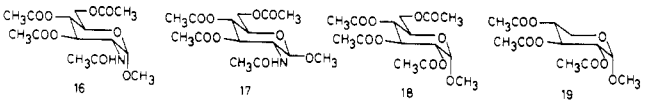
[a-f] Assignments may have to be interchanged. [g] Spectra of 1-3 were run in pyridine-$d_5$.

The aglycon methyl ester **4**, $C_{31}H_{50}O_3$, 1730 $cm^{-1}$, has a hydroxyl group (3620 $cm^{-1}$) and exo methylene double bonds (1640 and 900 $cm^{-1}$). The $^1H$ NMR spectrum of **4** showed the presence of four quaternary methyl signals at $\delta$ 0.68, 0.79, 0.82, and 1.00, two vinylic methyl signals at $\delta$ 1.74 and 1.78, and one methoxyl signal at $\delta$ 3.68. Signals due to the vinyl protons appeared at $\delta$ 4.58, 4.80, 4.84, and 5.40 as broad peaks. These $^1H$ NMR assignments were very consistent with the corresponding $^{13}C$ NMR data. Further correlation of the $^{13}C$ NMR spectrum of **4** with those of lansic acid (**5**) and $\alpha$-onocerin derivatives **6** and **13**[8] allowed us to deduce the possible structure **4** for this aglycon (see Tables I and II). The stereochemistry at C-3 was assigned to be $\beta$-hydroxyl from the coupling parameters of the carbinyl proton ($\delta$ 3.28, dd, $J$ = 11 and 4 Hz). The relative position of the two double bonds ($\delta$ 148.2 s, 106.7 t, and 136.0 s, 121.7 d), other than the isopropenyl group, was determined to be at $\Delta^{8(26)}$ and $\Delta^{14(15)}$, respectively, by the careful examination of LIS–NMR, in which signals due to the C-26 methylene protons ($\delta$ 4.58 and 4.84) moved downfield ($\Delta\delta$ 0.83 and 0.99) to a greater extent than that of the C-15 proton ($\delta$ 5.40, $\Delta\delta$ 0.12) on the addition of 0.5 equiv of $Eu(fod)_3$.

Chart II




12, R = O
14, R = NOH




Jones' oxidation of **4** provided ketone **12** in 86% yield (Chart II). The CD spectrum of this ketone in methanol

(7) Bock, K.; Pederson, C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293.
(8) We thank Dr. T. Harayama of Kyoto University for the generous gift of $\alpha$-onocerin.

Table II. $^1$H NMR Spectral Data (100 or 200 MHz, in $CDCl_3$ or Pyridine-$d_5$)

| compd | spectral data |
|---|---|
| 1[b] | 0.62, 0.83, 0.89, 1.05, 1.80, 1.82, 2.10 (each 3 H, s), 3.20–4.00 (m), 4.63 (1 H, br s), 4.84 (3 H, br), 5.33 (1 H, br), 6.30 (br), 8.58 (1 H, br) |
| 2[b] | 0.75, 0.93, 0.98, 1.29 (each 3 H, s), 1.83 (6 H, s), 3.62–4.48 (6 H, complex), 4.77 (1 H, br s), 4.88 (1 H, br), 4.90 (2 H, br), 5.40 (1 H, br), 5.80 (5 H, br) |
| 3[b] | 0.73, 0.89, 0.95, 1.26 (each 3 H, s), 1.78 (6 H, s), 3.80–4.36 (5 H, complex), 4.72 (1 H, br), 4.88 (2 H, br), 5.36 (1 H, br) |
| 4[a] | 0.68, 0.79, 0.82, 1.00, 1.74, 1.78, 3.68 (each 3 H, s), 3.28 (1 H, dd, $J$ = 11, 4 Hz), 4.58 (1 H, br), 4.80 (1 (1 H, br), 4.84 (2 H, br), 5.40 (1 H, br) |
| 7[a] | 0.67, 0.76, 0.82, 0.92, 1.73, 1.78, 1.93, 2.04, 2.05, 2.09, 3.67 (each 3 H, s), 3.12 (1 H, dd, $J$ = 10, 4 Hz), 3.68 (1 H, m), 3.93 (1 H, q, $J$ = 8 Hz), 4.13 (1 H, dd, $J$ = 13, 3 Hz), 4.28 (1 H, dd, $J$ = 12, 5 Hz), 4.57 (1 H, br), 4.67 (1 H, d, $J$ = 8 Hz), 4.79 (1 H, br), 4.85 (2 H, br), 5.07 (1 H, t, $J$ = 8 Hz), 5.28 (1 H, t, $J$ = 8 Hz), 5.39 (1 H, br), 5.56 (1 H, d, $J$ = 8 Hz) |
| 8[a] | 0.67, 0.73, 0.82, 0.92, 1.74, 1.78, 2.01, 2.04, 2.05, 2.09, 3.67 (each 3 H, s), 3.15 (1 H, dd, $J$ = 11, 4 Hz), 3.70 (1 H, m), 4.14 (1 H, dd, $J$ = 12, 3 Hz), 4.30 (1 H, dd, $J$ = 12, 5 Hz), 4.56 (1 H, d, $J$ = 8 Hz), 4.57 (1 H, br), 4.80 (1 H, br), 4.85 (2 H, br), 5.06 (1 H, t, $J$ = 8 Hz), 5.14 (1 H, t, $J$ = 8 Hz), 5.23 (1 H, t, $J$ = 8 Hz), 5.40 (1 H, br) |
| 9[a] | 0.67, 0.73, 0.81, 0.92, 1.74, 1.78, 2.04, 2.05, 2.06, 3.67 (each 3 H, s), 3.12 (1 H, dd, $J$ = 11, 4 Hz), 3.32 (1 H, dd, $J$ = 12, 8 Hz), 4.12 (1 H, dd, $J$ = 12, 5 Hz), 4.53 (1 H, d, $J$ = 8 Hz), 4.57 (1 H, br), 4.80 (1 H, br), 4.84 (2 H, br), 4.98 (1 H, t, $J$ = 8 Hz), 4.99 (1 H, t, $J$ = 8 Hz), 5.20 (1 H, t, $J$ = 8 Hz), 5.40 (1 H, br) |
| 12 | 0.82, 0.85, 1.03, 1.10, 1.76, 1.80, 3.63 (each 3 H, s), 4.62 (1 H, br), 4.78 (2 H, br), 4.90 (1 H, br), 5.36 (1 H, br) |

[a] Spectra run at 200 MHz. [b] Spectra run in pyridine-$d_5$.

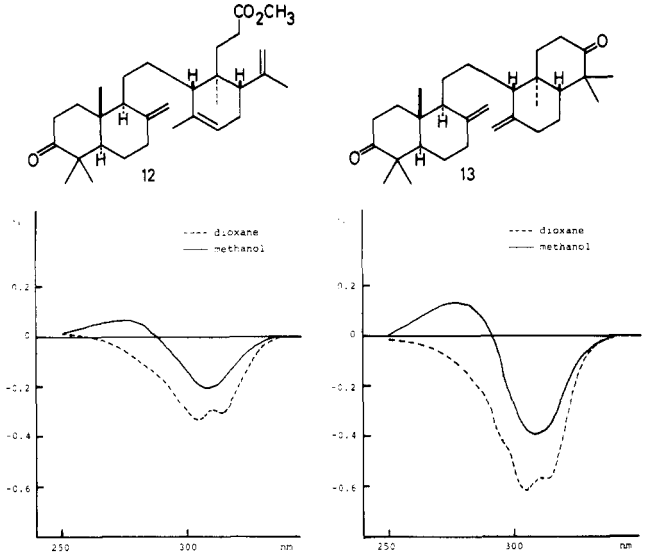



**Figure 1.** CD spectra of **12** and **13**.

exhibited a maximum at 275 nm ($\Delta\epsilon$ +0.06) and a minimum at 307 nm ($\Delta\epsilon$ −0.21). Both of the CD extrema appeared at the same region, but in approximately half magnitude, compared to those of $\alpha$-onoceradione (**13**) (Figure 1). This fact indicates that the ketone **12** includes a structural unit corresponding to half of the symmetric $\alpha$-onoceradione molecule and that the absolute configuration of **12** is the same that of the latter compound, assuming that **4** was biosynthesized from an onocerane-type precursor by oxidative cleavage of one decalin system selectively.

The final confirmation of the basic carbon skeleton and absolute configuration of the aglycon was carried out by the chemical correlation of the ketone **12** with lansic acid (**5**) in the following way. The ketone **12** was transformed to oxime **14** in 93% yield, which was further changed to nitrile **15** in 30% yield on treatment with methanesulfonyl chloride in pyridine.[10] Hydrolysis of **15** with sodium hydroxide gave an acid (**5**) in 36% yield. This product was identical in all respects, including optical rotation, with the natural lansic acid. Thus, the full structure of lansioside A is given by the formula **1**, including the absolute configuration.

Lansiosides B (**2**), an amorphous solid, and C (**3**), mp 124.5–126 °C, $C_{35}H_{56}O_7 \cdot {}^1/_2H_2O$, were other related glycosides with the same aglycon. Acid-catalyzed methanolysis of lansiosides B and C gave the aglycon methyl ester **4**[11] and anomeric mixtures of methyl glycosides. Upon acetylation of the latter mixture, followed by careful purification, methyl $\alpha$-D-glucopyranoside tetraacetate (**18**)[12] was isolated from lansioside B (**2**) and methyl $\alpha$-D-xylopyranoside triacetate (**19**)[13] from lansioside C (**3**). Both of the sugar derivatives were fully identified with the authentic samples. The stereochemistry at anomeric carbons of **2** and **3** were determined by NMR analysis. The parameters for the anomeric protons and carbons [$\delta$ 4.67 (d, $J$ = 8 Hz) and 103.1 d for lansioside B methyl ester tetraacetate (**8**) and $\delta$ 4.56 (d, $J$ = 8 Hz) and 103.0 d for lansioside C methyl ester triacetate (**9**)] clearly showed the $\beta$-glycosidyl structures. Therefore, the full structures of lansiosides B and C were established as shown in the formulas **2** and **3**, respectively, including the absolute configurations.

The aglycon **10** showed a very close $R_f$ value on TLC to that of lansic acid (**5**) with various solvent systems. However, their methyl esters were chromatographically separable from each other. In this way, natural lansic acid (**5**) was found to be originally accompanied by the aglycon acid **10** of lansiosides, which is now named lansiolic acid.

Since lansioside A is a structurally unique triterpene glycoside with a novel amino sugar and the natural abundance of lansiosides is comparatively excellent, we have submitted these compounds to a variety of biological tests. Particularly remarkable was the in vitro inhibitory effect of lansioside A on the leukotriene $D_4$ ($LTD_4$) induced contraction[14] of guinea pig ileum. Lansioside A (**1**) in-

(9) Tsuda, Y.; Sano, T. *Chem. Pharm. Bull.* **1980**, *28*, 3134. Professor Y. Tsuda Kindly sent us the CD spectrum of **13**. See also: Hanna, R.; Levisalles, J.; Ourisson, G. *Bull. Soc. Chim. Fr.* **1960**, 1938.

(10) Whitman, G. H. *J. Chem. Soc.* **1960**, 2016.

(11) Prolonged reflux (~10 h), which gave a higher yield of methyl glycoside, causes partial isomerization of the double bonds of the triterpene moiety.

(12) Inoue, Y.; Onodera, K.; Karasawa, I.; Nishisawa, Y. *J. Agric. Chem. Jpn.* **1951**, *25*, 499.

(13) Jennings, H. J. *Can. J. Chem.* **1971**, *49*, 1355.

hibited contraction in a dose-dependent manner at a concentration of $10^{-9}$ M $LTD_4$ ($IC_{50}$ $2.4 \times 10^{-6}$ g/mL).[15] Lansiosides B (**2**) and C (**3**) were approximately 10 times less potent and lansiolic acid (**10**) was inactive. This activity of lansiosides was potent indeed, but nonspecific at higher concentration ($10^{-5}$ g/mL), and also applicable to histamine- or serotonin-induced contraction of the ileum system.

## Experimental Section

**General Procedures.** Infrared (IR) spectra were recorded on a JASCO IRA-1 spectrometer. $^1H$ NMR spectra were obtained on either 100-MHz (JEOL PS-100 or FX-100) or 200-MHz (JEOL FX-200) instruments. $^{13}C$ NMR spectra were recorded on 25-MHz (JEOL FX-100) instrument using a $^1H/^{13}C$ dual probe. The chemical shifts are reported in $\delta$ units relative to internal tetramethylsilane ($\delta = 0$). Mass spectra were determined at 30 eV on a JEOL JMS D-300 spectrometer. For GC–MS analysis, a 2 mm × 2 m column of 3% silicon OV-1 on Chromosorb W AW was employed. The optical rotation was recorded on a JASCO DIP-4 digital polarimeter in the solvent and concentration (1-dm cell) indicated. CD spectra were determined on a JASCO J-20 automatic recording Spectro-Polarimeter. Analytical TLC was carried out on precoated plates of Merck silica gel 60 $F_{254}$. Column chromatography was performed on Merck silica gel 60 (Art. 7734, 70–230 mesh).

**Isolation of Lansiosides.** The dried peel of *Lansium domesticum* Jack var. Duku (2 kg, collected at Bogor, Indonesia) was crushed with ethanol (15 L), and the resulting mixture was allowed to stand at room temperature for 2 weeks. The filtrate was concentrated to ca. 0.5 L, and water (3 L) was added. This mixture was extracted with dichloromethane (500 mL, three times), and the combined extracts were dried over sodium sulfate. Concentration of the filtrate gave a gummy syrup (250 g). This syrup (55 g) was subjected to column chromatography on silica gel (500 g) with dichloromethane as the first eluent. The first fraction (a, dichloromethane 2 L) provided a mixture of less polar materials (8.1 g), and the following fraction (b, 95:5 mixture of dichloromethane and ethanol) gave a mixture of lansic acid (**5**) and lansiolic acid (**10**) (28 g). Fraction *c* (1.11 g, 90:10 mixture of dichloromethane and ethanol), fraction d (1.76 g, 85:15), and fraction e (1.80 g, 80:20) were successively collected in this order.

The fraction e (1.80 g) was further purified by silica gel column chromatography (80 g, 4:1 mixture of dichloromethane and ethanol) to give lansioside A (**1**, 737 mg, 0.36% yield) as crystalline solid. Repeated recrystallization from ethanol afforded the analytical sample: mp 174–175 °C; $[\alpha]^{15}_D$ +26.5° (*c* 1.06, ethanol); IR (KBr) 3400, 1705, 1640, 1560, 890 $cm^{-1}$. Anal. Calcd for $C_{38}H_{61}NO_8 \cdot H_2O$: C, 67.33; H, 9.37; N, 2.07. Found: C, 67.00; H, 9.33; N, 2.08.

Fraction d (1.76 g) was chromatographed on a silica gel column (100 g, 7:1 mixture of dichloromethane and ethanol) to give lansioside B (**2**, 611 mg, 0.27% yield) as an amorphous solid: $[\alpha]^{23}_D$ +12.5° (*c* 1.50, ethanol); IR ($CHCl_3$) 3400, 1705, 1640, 890 $cm^{-1}$.

Fraction c (1.11 g) was purified by silica gel column chromatography (110 g, 8:1 mixture of dichloromethane and ethanol), providing lansioside C (**3**, 385 mg, 0.17% yield) as an amorphous solid. Crystallization from chloroform–hexane afforded an analytical sample: mp 124.5–126 °C; $[\alpha]^{24}_D$ +12.4° (*c* 0.79, ethanol); IR ($CHCl_3$) 3400, 1705, 1640, 890 $cm^{-1}$. Anal. Calcd for $C_{35}H_{56}O_7 \cdot {}^1/_2H_2O$: C, 70.32; H, 9.61. Found: C, 70.06; H, 9.64.

**Lansioside A Methyl Ester Triacetate (7).** A mixture of lansioside A (**1**, 120 mg), pyridine (4 mL), and acetic anhydride (3 mL) was stirred at room temperature for 48 h. The volatile materials were distilled off under reduced pressure, and the residual syrup was dissolved in ether (1 mL). To this solution was added an excess of diazomethane in ether. After 30 min, volatile materials were evaporated in vacuo, and the residue was chromatographed on silica gel column (25 g, 5:2 mixture of ethyl acetate and hexane) to give **7** (148 mg, 93% yield) as a colorless syrup. Crystallization from a mixture of benzene and hexane provided an analytical sample: mp 149–150 °C; $[\alpha]^{14}_D$ +15.1° (*c* 1.10, $CHCl_3$); IR ($CHCl_3$) 1740, 1680, 1505, 890 $cm^{-1}$. Anal. Calcd for for $C_{45}H_{69}NO_{11}$: C, 67.56; H, 8.69; N, 1.75. Found: C, 67.50; H, 8,87; N, 1.70.

**Lansioside B Methyl Ester Tetraacetate (8).** Lansioside B (**2**, 212 mg) was converted to a methyl ester tetraacetate **8** (158 mg) by using procedure similar to that described above. Repeated recrystallization from ether and petroleum ether gave an analytical sample: mp 111.5–112.5 °C; $[\alpha]^{17}_D$ +17.8° (*c* 1.22, $CHCl_3$); IR ($CHCl_3$) 1740, 1635, 890 $cm^{-1}$. Anal. Calcd for $C_{45}H_{68}O_{12}$: C, 67.48; H, 8.56. Found: C, 67.44; H, 8.60.

**Lansioside C Methyl Ester Triacetate (9).** By the same method, lansioside C (**3**, 44 mg) was converted to methyl ester triacetate **9** (52 mg). Recrystallization from ether and petroleum ether afforded an analytical sample: mp 131–132 °C; $[\alpha]^{18}_D$ +6.2° (*c* 0.50, $CHCl_3$); IR ($CHCl_3$) 1730, 1630, 880 $cm^{-1}$. Anal. Calcd for $C_{42}H_{64}O_{10}$: C, 69.20; H, 8.85. Found: C, 69.32; H, 8.96.

**Methanolysis of Lansioside A (1).** A solution of lansioside A (**1**, 360 mg) and concentrated sulfuric acid (25 mg) in methanol (15 mL) was heated at reflux under an argon atmosphere for 2 h. After addition of water (40 mL), methanol was removed under vacuum. The resulting mixture was extracted with ether three times, and the combined organic extract was dried and concentrated. The gummy residue was subjected to column chromatography on silica gel (25 g, 5:1 mixture of hexane and ethyl acetate) to give **4** (157 mg, 62%) as a colorless syrup: $[\alpha]^{19}_D$ +34.9° (*c* 1.36, $CHCl_3$); IR ($CHCl_3$) 3620, 1730, 1640, 900 $cm^{-1}$; high-resolution mass spectrum calcd from *m/e* $C_{31}H_{50}O_3$, 470.3759, found 470.3780.

The aqueous phase of the extraction was neutralized by using Amberlite IRA-430 and then concentrated to dryness under vacuum. The resulting syrup was dissolved in pyridine (2 mL) and acetic anhydride (2 mL), and the mixture was stirred at room temperature for 19 h. The volatile materials were evaporated under vacuum, and silica gel column chromatography of the residue using ethyl acetate gave pure methyl 2-acetamido-2-deoxy-$\alpha$-D-glucopyranoside triacetate (**16**, 16 mg) and its $\beta$-anomer **17** (73 mg). Recrystallization of these products from ethyl acetate provided an analytical sample of **16** [mp 112–114 °C (lit. mp 114–115 °C); $[\alpha]^{20}_D$ +84.6° (*c* 0.79, $CHCl_3$) (lit.[5] $[\alpha]^{22}_D$ +94.3° (*c* 1.06, $CHCl_3$)] and **17** [mp 156–157 °C (lit. mp 163–164 °C); $[\alpha]^{18}_D$ −6.4° (*c* 1.25, $CHCl_3$) (lit.[5] $[\alpha]^{23}_D$ −10.2° (*c* 1.01, $CHCl_3$))]. Both of the sugar derivatives gave identical IR and NMR ($^1H$ and $^{13}C$) spectra with those of authentic materials.

**Methanolysis of Lansioside B (2).** Lansioside B (112 mg) was treated by the same procedure as described above, and the aglycon ester **4** (47 mg) was obtained from the ether-soluble portion. The neutralized aqueous phase was concentrated under vacuum and following treatment of the residue with pyridine (2 mL) and acetic anhydride (1.5 mL) gave a mixture of methyl glucoside tetraacetates. Column chromatography on silica gel (20 g) using a 3:2 mixture of petroleum ether and ether gave a mixture of $\alpha$- and $\beta$-methyl glucoside tetraacetate (23 mg) and pure $\alpha$-glucoside **18** (16.2 mg). The latter was crystallized from ethanol to give an analytical sample: mp 97–98 °C (lit.[12] mp 100 °C); $[\alpha]^{22}_D$ +116.5° (*c* 0.81, $CHCl_3$) (lit.[12] $[\alpha]^{22}_D$ +131.2° ($CHCl_3$)); IR and NMR ($^1H$ and $^{13}C$) spectra were identical with those of authentic material.

**Methanolysis of Lansioside C (3).** Lansioside C (**3**, 249 mg) was treated as above to give **4** (123 mg) from the ether-soluble fraction. The aqueous phase was neutralized and concentrated. Acetylation of the residue followed by column chromatography on silica gel (30 g) using a 3:2 mixture of petroleum ether and ether gave pure methyl $\alpha$-D-xylopyranoside triacetate (**19**, 21 mg) as a crystalline solid: $[\alpha]^{22}_D$ +121.8° (*c* 1.00, $CHCl_3$) (lit.[13] $[\alpha]_D$ +118° (*c* 3.7, $CHCl_3$)); IR and NMR spectra were identical with those of authentic sample.

**Keto Ester 12.** To a solution of **4** (110 mg, 0.23 mmol) in acetone (3 mL) was added Jones' reagent (0.21 mL, 0.36 mmol) dropwise, and the mixture was stirred at 0 °C for 30 min. The excess oxidizing agent was destroyed by the addition of 2-propanol

(14) Leukotriene $D_4$, a novel peptidolipid, is known to be a Slow Reacting Substances of Anaphylaxis (SRS-A) and to play an important role in hypersensitivity reactions such as asthma. See: Hammarström, S.; Samuelsson, B. *Biochem. Biophys. Res. Commun.* **1980**, *92*, 946. Samuelsson, B.; Hammarström, S. *Prostaglandins* **1980**, *19*, 645.

(15) This biological examination was conducted by Dr. Tsumoru Miyamoto of Ono Pharmceu. Co. Ltd. Detailed experimental procedures will be presented elsewhere.

(0.2 mL), and then water (20 mL) was added. The ether extract was dried and concentrated. Column chromatography of the residue on silica gel (8 g) using a 7:1 mixture of petroleum ether and ether gave pure ketone **12** (91 mg, 86% yield) as a colorless syrup. Recrystallization from hexane gave an analytical sample: mp 91–92 °C; $[\alpha]^{17.5}_D$ +35.2° (*c* 0.81, $CHCl_3$); IR ($CHCl_3$) 1740, 1700, 1640, 900 $cm^{-1}$. Anal. Calcd for $C_{31}H_{48}O_3$: C, 76.44; H, 10.32. Found: C, 79.28; H, 10.39.

**Oxime 14.** A solution of ketone **12** (180 mg, 0.38 mmol) and hydroxyamine hydrochloride (80 mg, 1.15 mmol) in a 1:1 mixture of pyridine and ethanol (3 mL) was refluxed for 2 h under an argon atmosphere. To the cooled mixture was added brine (20 mL), and following ethyl acetate extraction, the mixture afforded a crude product. Column chromatography on silica gel (15 g) using a 6:1 mixture of petroleum ether and ether gave oxime **14** (173 mg, 93% yield) as a colorless syrup: $[\alpha]^{16}_D$ −1.71° (*c* 1.46, $CHCl_3$); IR ($CHCl_3$) 3600, 3300, 1740, 1640, 890 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 0.80 (6 H, s), 1.05 (3 H, s), 1.17 (3 H, s), 1.76 (3 H, s), 1.78 (3 H, s), 3.64 (3 H, s), 4.58 (1 H, br s), 4.76 (2 H, m), 4.84 (1 H, br s), 5.29 (1 H, br); high-resolution mass spectrum calcd for $C_{31}H_{49}NO_3$ 483.3715, found 483.3720 ($M^+$).

**Nitrile 15.** To a solution of oxime **14** (75 mg, 0.16 mmol) in pyridine (2 mL) was added methanesulfonyl chloride (53 mg, 0.47 mmol) dropwise, and the mixture was stirred at 0 °C for 5 h. Evaporation of the volatile materials under reduced pressure and column chromatography on silica gel (15 g) using a 10:1 mixture of petroleum ether and ether afforded pure nitrile **15** (22 mg, 30% yield) as a colorless syrup; $[\alpha]^{16}_D$ +25.2° (*c* 1.06, $CHCl_3$); IR ($CHCl_3$) 3060, 2270, 1740, 1640, 900 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 0.74 (3 H, s), 0.83 (3 H, s), 1.73 (3 H, s), 1.78 (6 H, s), 3.68 (3 H, s), 4.61 (1 H, br), 4.70 (1 H, br), 4.80 (2 H, m), 4.90 (2 H, br), 5.38 (1 H, br); high-resolution mass spectrum calcd for $C_{31}H_{47}NO_2$ 465.3609, found 465.3580 ($M^+$).

**Lansic Acid (5).** The nitrile **15** (36 mg, 0.08 mmol) was dissolved in a 20% ethanol solution of potassium hydroxide (5 mL), and the mixture was refluxed for 3 h under an argon atmosphere. After addition of brine (15 mL), the mixture was acidified with 5% HCl. Ethyl acetate extraction and column chromatography on silica gel (10 g) using hexane and ethyl acetate (3:1) afforded lansic acid (**5**, 13 mg, 36% yield) as a crystalline solid. This product was identical with authentic material in all respects, including optical rotation.

**Acknowledgment.** We are indebted to Drs. N. Hamanaka and T. Miyamoto of Ono Pharmaceu. Co. Ltd. for the determinations of 200-MHz NMR, high-resolution mass spectra, and the biological examinations of lansiosides and to Professor Y. Tsuda of Kanazawa University for a helpful discussion. This work was supported by the research grants of The Naito Foundation (1980, 1981), The Toyota Foundation (1981), and the Suntory Institute for Bioorganic Research (1980) and the Grant-in-Aid for Special Project Research (No. 57218020, 1982) and the Grant-in-Aid for Scientific Research (No. 57470026, 1982) of the Ministry of Education, Japanese Government.

**Registry No.** **1**, 82537-86-8; **2**, 87453-34-7; **3**, 87453-35-8; **4**, 87453-36-9; **5**, 19954-99-5; **6**, 511-01-3; **7**, 87453-37-0; **8**, 87453-38-1; **9**, 87453-39-2; **10**, 87453-40-5; **12**, 87453-41-6; **13**, 6929-24-4; **14**, 87453-42-7; **15**, 87453-43-8; **16**, 2595-39-3; **17**, 2771-48-4; **18**, 604-70-6; **19**, 20880-54-0.

# Insertion of Oxygen into C–P Bonds of Some Strained Phosphorus Heterocycles[1]

Louis D. Quin,* John C. Kisalus, and Keith A. Mesch

*Gross Chemical Laboratory, Duke University, Durham, North Carolina 27706*

*Received May 12, 1983*

Cyclic phosphine oxides with exceptionally contracted internal C–P–C angles have been found to undergo oxygen insertion into a C–P bond on treatment with peracids. The reaction is of synthetic value in creating 1,2-oxaphospha ring systems. It has been applied to two phosphetane oxides and the bridged compounds 5-isopropyl-2,6-dimethyl-6-phosphabicyclo[3.1.1]hept-2-ene 6-oxide and 4-methyl-4-phosphatetracyclo-[3.3.0.$0^{2,8}$.$0^{3,6}$]octane 4-oxide. Reaction with the former was regiospecific, insertion occurring at the least substituted carbon. The 7-phosphanorbornane moiety in the product of hydrogenation of a phosphole oxide dimer also underwent the ring expansion, providing a 1:1 mixture of the two possible insertion products. Some new insertion products from phosphole oxide dimers were also prepared. All new oxaphospha derivatives were characterized by $^1H$, $^{31}P$, and $^{13}C$ NMR spectroscopy.

The carbon–phosphorus bond in phosphoryl compounds is generally resistant to oxidizing agents, and many oxidation reactions of organic chemistry can be safely performed on carbon functional groups without involvement of the phosphorus function. Epoxidation of double bonds falls in this category, and conversions of unsaturated phosphonic or phosphinic acids or esters, and of phosphine oxides, into epoxy derivatives are known .[2] It was therefore of some significance when oxygen insertion into a C–P bond was noted[3] in the case of unsaturated phosphine oxides of the 7-phosphanorbornene[2] and the 8-phosphabicyclo[3.2.1]octene[4] systems. Kashman and Awerbouch observed[3] that a C–P bond of the 7-phosphanorbornene moiety of the phosphole oxide dimer structure **1** was attacked by *m*-chloroperbenzoic acid (MCPBA) in preference to the double bond. Of the two possible oxygen-insertion products, only **2** was obtained (75%); its structure was proposed from its $^1H$ NMR properties. Continued exposure of **2** to the peracid then provided the epoxy derivative **3**. The stability of the initial product **2** was poor, and it decomposed at 25 °C with a half-life of 3 h by retrocycloaddition to form a dihydrophosphindole **4** and a polymer of phenylmetaphosphonic acid. A similar insertion was noted for the phosphabicyclooctene system.[4]

Phosphole oxide dimers are unique in other regards, and a study of these compounds is presently in progress in this laboratory.[5] With several compounds on hand, we pro-

(1) Presented at the 184th National Meeting of the American Chemical Society, Kansas City, MO, Sept 15, 1982; ORGN 110.

(2) See, for example, Quin, L. D.; Symmes, C., Jr.; Middlemas, E. D.; Lawson, H. F. *J. Org. Chem.* **1980**, *45*, 9688.

(3) Kashman, Y.; Awerbouch, O. *Tetrahedron* **1975**, *31*, 53.

(4) Kashman, Y.; Awerbouch, O. *Tetrahedron* **1975**, *31*, 45.

0022-3263/83/1948-4466$01.50/0 