Phytochemistry, 1973, Vol. 12, pp. 2737 to 2739. Pergamon Press. Printed in England.

Δ^{16}-DEHYDROADYNERIGENIN GLYCOSIDES OF *NERIUM ODORUM**

TATSUO YAMAUCHI, YŪJIRŌ MŌRI and YASUKO OGATA
Faculty of Pharmaceutical Sciences, Fukuoka University, Fukuoka, Japan

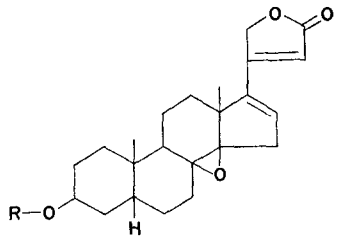
(*Received* 26 *March* 1973. *Accepted* 2 *July* 1973)

Key Word Index—*Nerium odorum*; Apocynaceae; oleander; cardenolides.

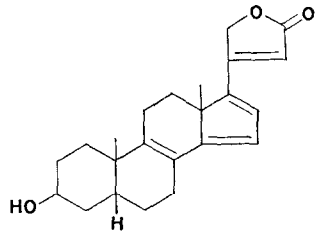
Abstract—β-D-Diginoside and β-D-digitaloside of Δ^{16}-dehydroadynerigenin were isolated from the oven dried leaves of *Nerium odorum*.

INTRODUCTION

THE LEAVES of *Nerium oleander* and *N. odorum* are known to contain many cardioactive glycosides.[1] Previously, the authors obtained oleandrin, together with odorosid-*A* (digitoxigenin β-D-diginoside), adynerin (adynerigenin β-D-diginoside)[2] and an unknown compound (I) from fresh leaves of *N. odorum* which had been dried in the oven over 80°.[3] A more polar substance (II), having the same colour reactions as I and similar behaviour to odorosid H on TLC and column chromatography, was isolated as a minor constituent. This paper deals with the structural elucidation of I and II as Δ^{16}-dehydroadynerigenin glycosides.



(I) R = -β-D-diginosyl
(II) R = -β-D-digitalosyl
(III) R = H



(IV)

RESULTS AND DISCUSSION

I, m.p. 188–190°, $[\alpha]_D$ +71·4°, isolated from the mother liquor from the crystallization of oleandrin following column chromatography, gave positive Legal and Kedde tests. The presence of a double bond system conjugated with an α,β-unsaturated lactone

* Part III in the series '*Nerium*'. For Part II see YAMAUCHI, T., HARA, M. and MIHASHI, K. (1972) *Phytochemistry* **11**, 3345.

[1] NEUMANN, W. (1937) *Chem. Ber.* **70**, 1547; TSCHESCHE, R., CHAUDHURI, P. K. and SNATZKE, G. (1964) *Naturwissenschaften* **51**, 139; ISHIDATE, M. and TAMURA, Z. (1950) *Yakugaku Zasshi* **70**, 7; OKADA, M. (1952) *Yakugaku Zasshi* **73**, 86; TAKEMOTO, T. and KOMETANI, K (1954) *Yakugaku Zasshi* **74**, 1263.
[2] JANIAK, P. S., WEISS, E., EUW, J. V. and REICHSTEIN, T. (1963) *Helv. Chim. Acta* **46**, 374.
[3] YAMAUCHI, T. and EHARA, Y. (1972) *Yakugaku Zasshi* **92**, 155.

ring was suggested by the hypsochromic shift from 267 to 217 nm on oxidation with *m*-chloroperbenzoic acid. After mild hydrolysis, I gave, along with D-diginose, two aglycones. One of these (III), colourless needles with absorption at 267 nm seemed to be the true aglycone, the IR spectra of the acetates of both I and III showing no tertiary hydroxyl group. The second aglycone (IV), obtained as yellow prisms, absorbs at 387 nm, probably due to a triene system conjugated with the unsaturated lactone ring. Since the MS of III and IV gave a similar pattern with those of adynerigenin (3β-hydroxy-8,14β-epoxy-5β-carda-20:22-enolide) and its $\Delta^{8(9),14}$-diene compound (3β-hydroxy-5β-carda-8:9; 14:15; 20:22-trienolide)[2] (V) with a difference of 2 m.u. III, and IV were assumed to be Δ^{16}-dehydroadynerigenin and its $\Delta^{8(9),14,16}$-triene compound, respectively.

In order to confirm these structures, I and III were subjected to catalytic hydrogenation. Whereas Δ^{16}-14β-hydroxy cardenolides are known to be reduced to corresponding 17βH-cardenolides,[4] the physical constants of the reduction products were in good agreement in all respects with those of adynerin and adynerigenin, respectively. Hence, the structure of I was elucidated as Δ^{16}-dehydroadynerigenin-D-diginoside [3β-*O*-(D-diginosyl)-8,14β-epoxy-5β-carda-16:17; 20.22-dienolide], and its glycosidic linkage was deduced to be β-form according to the coupling constant of anomeric proton and the Klyne's rule as well as the fact that I was reduced to adynerin.

II was obtained from a more polar fraction than I. According to the spectral data, it appeared that II has the same aglycone as I. On refluxing with 0·05 N hydrochloric acid for 30 min, II was hydrolyzed partially to III and D-digitalose, and is therefore, the D-digitaloside of Δ^{16}-dehydroadynerigenin. The configuration of the sugar–aglycone link was assigned to the β-form from the molecular rotations of II and III, and NMR data.

Although Δ^{16}-cardenolides found from natural sources[5] have been regarded as artifacts from the process of extraction and isolation, Δ^{16}-dehydroadynerigenin can be obtained in the form of more polar glycosides than I or II in the carefully prepared extracts from fresh leaves of *N. odorum*.[3]

EXPERIMENTAL

TLC was developed on a Kiesel gel G, with $CHCl_3$–MeOH–H_2O (7:3:1, lower),[6] or hexane–EtOAc (1:3). PC for sugars was run with toluene–BuOH (1:9) mixture saturated with H_2O.[7] GLC was taken with 1·5% SE30 column at 240° of column temp.

Isolation of I *and* II. Fresh leaves of *N. odorum* were dried in the oven at 80°, powdered, and percolated with MeOH. The percolate was concentrated, diluted with H_2O to 50% and filtered. The filtrate was extracted with C_6H_6 and the C_6H_6 extracts were crystallized from EtOAc–hexane to give oleandrin. The mother liquor of the crystallization was subjected to column chromatography on silica gel ($\times 50 \sim 100$) with C_6H_6–acetone (15:1 to 5:1) as an eluting solvent. I was obtained with 15:1 C_6H_6–acetone elution, prior to oleandrin, while II was afforded with 5:1 mixture after elution of odorosid H. Each substance was purified through successive column chromatography following recrystallization from EtOAc–hexane (for I, yield 0·032%) or from C_6H_6–acetone (for II, 0·0016%).

Physical constants of I. I: m.p. 188–190°, $[\alpha]_D^{20}$ +71·4° in MeOH (*c* 0·37), $[M]_D$ +367° ($[M]_{D\,I} - [M]_{D\,III} = -41°$, Me-$\alpha$-D-diginoside: +142° ± 3°, Me-β-D-diginoside: −200° ± 50°), UV: λ_{max}^{MeOH} 267 nm (ϵ 17 100). (Found: C, 70·1; H, 8·3. $C_{30}H_{42}O_7$ requires: C, 70·0; H, 8·2%). IR: ν_{max}^{KBr} cm^{-1} 3580, 2990, 2920, 1800,

[4] SATOH, D. and ISHII, H. (1960) *Yakugaku Zasshi* **80,** 1143.

[5] AEBI, A. and REICHSTEIN, T. (1950) *Helv. Chim. Acta* **33,** 1013; BERTHOLD, R., WEHRLI, W. and REICHSTEIN, T. (1965) *Helv. Chim. Acta* **48,** 1659; KAUFMANN, H., WEHRLI, W. and REICHSTEIN, T. (1965) *Helv. Chim. Acta* **48,** 65, 83; PATNAIK, G. K., VOHRA, M. M., KAPIL, R. S and ANAND, N. (1966) *J. Pharm. Sci.* **55.** 1425; DOSKOTCH, R. W., MALIK, M. Y., HUFFORD, C. C., MALIK, S. N., TRENT, J. E. and KUBELKA, W. (1972) *J. Pharm. Sci.* **61,** 570.

[6] KAWASAKI, T. and MIYAHARA, K. (1963) *Chem. Pharm. Bull. (Tokyo)* **11,** 1546.

[7] RENKONEN, O. and SCHINDLER, O. (1956) *Helv Chim. Acta* **39,** 1490.

1750, 1640. MS: (*m/e*) 514 (M^+), NMR: δ (in $CDCl_3$), 1·02 (3H, *s*), 1·20 (3H, *s*), 1·30 (3H, *d*), 2·55 (2H, *d*), 3·40 (3H, *s*), 3·67 (1H, *m*), 4·02 (1H, *m*), 4·45 (1H, *dd*, 9Hz, anomeric proton of D-diginose), 4·92 (2H, *s*), 5·92 (1H, *s*), 6·05 (1H, *m*). Keller–Kiliani test, Legal test, Kedde test and Baljet test were all positive. Intense yellow colour and yellow fluorescence were observed with $SbCl_3$ reagent on TLC plate under natural- and UV-light, respectively. *I-Acetate.* m.p. 125–130°, $[\alpha]_D^{20}$ +36·4° in EtOH (*c* 0·22), IR: ν_{max}^{KBr} cm^{-1} 3000, 2960, 1800, 1780, 1640. No absorption between 4000 and 3000 cm^{-1} was observed. MS: (*m/e* 556 (M^+). *I-Epoxide.* To I (100 mg) in $CHCl_3$, 260 mg of *m*-chloroperbenzoic acid was added and allowed to stand overnight at room temp. The mixture was washed with aq. $NaHCO_3$, and $CHCl_3$ was evaporated *in vacuo*. The residue was purified through silica gel column, UV: λ_{max}^{MeOH} 220 nm (ϵ 12 600).

Hydrolysis of I. (a) I (400 mg) was heated with 20 ml of 0·05 N HCl–50% EtOH for 5 min and EtOH was evaporated *in vacuo*. The residual mixture was diluted with H_2O and the precipitate purified on a silica gel column with C_6H_6–acetone (15:1) mixture. Major product was crystallized from dil. EtOH to give 50 mg of prisms (III), m.p. 182–184°, $[\alpha]_D^{20}$ +110·2° in MeOH (*c* 0·59), $[M]_D$ +408°, UV: λ_{max}^{MeOH} 267 nm (ϵ 18 900). MS: (*m/e*) 370·2151 (M^+, $C_{23}H_{30}O_4$ requires: 370·2144), 355, 311 (adynerigenin: 372 (M^+), 357, 313), IR: ν_{max}^{KBr} cm^{-1} 3400, 3055, 2950, 1782, 1750, 1733, 1625. NMR: δ (in $CDCl_3$) 1·15 (3H, *s*), 1·32 (3H, *s*), 2·66 (2H, *d*), 4·18 (1H, *m*), 4·99 (2H, *d*), 5·96 (1H, *s*), 6 11 (1H, *t*). *A-I-Acetate.* m.p. 195–196°, $[\alpha]_D^{20}$ +110·4° in MeOH (*c* 0·50), MS: (*m/e*) 412·2220 (M^+, $C_{25}H_{32}O_5$ requires: 412·2249). IR: ν_{max}^{KBr} cm^{-1} 3092, 1780, 1750 (sh), 1745, 1720, 1630. (b) I (80 mg) was refluxed with 8 ml of 0·05 N H_2SO_4–50% MeOH for 45 min, and worked up in the usual manner. IV crystallized to long prisms from dil. EtOH m.p. 192–193°, $[\alpha]_D^{20}$ +367·3° in MeOH (*c* 0·27). UV: λ_{max}^{MeOH} 387 nm (ϵ 19 800). MS: (*m/e*) 352·1986 (M^+, $C_{23}H_{28}O_3$ requires: 352·2038), 337, 319, 293, 280 (V: 354 (M^+), 339, 321, 295, 282). NMR: δ (in $CDCl_3$) 1·10 (3H, *s*), (1·20 (3H, *s*), 3·90 (1H, *m*), 5·03 (2H, *s*), 5·80 (1H, *s*), 6·15 (1H, *s*), 6·75 (1H, *s*). Water layer of the hydrolysis was used for the detection of sugar after treating in the usual manner. PC: $R_{rhamnose}$ 2·96 (D-diginose: 2·96; L-oleandrose: 3·27; D-digitalose: 2·23; L-rhamnose: 1·00).

Catalytic hydrogenation of I. I (100 mg) in EtOH) was shaken with 100 mg of Pd–C under H_2 for 30 min and the product was crystallized from MeOH to give dihydro-I as prisms, m.p. 218–222°, $[\alpha]_D^{20}$ +12·7° in MeOH (*c* 0·20). UV: λ_{max}^{EtOH} 217 nm (ϵ 12 000). It was in good agreement on direct comparison with authentic adynerin (m.p. 216–219°, $[\alpha]_D^{20}$ +15·0° in MeOH) (m.m.p., IR, TLC, ORD). On hydrogenation of III under the same condition with that of I, dihydro-compound was crystallized from MeOH to give prisms, m.p. 247–248°, $[\alpha]_D^{20}$ +31·3° in MeOH (*c* 0·20), UV: λ_{max}^{MeOH} 217 nm (ϵ 16 600), and its acetate was obtained in the usual manner, m.p. 174–175°, $[\alpha]_D^{20}$ +27·5° in MeOH (*c* 0 08). Both the dihydro-III and its acetate were in good agreement on direct comparison with adynerigenin (m.p. 241–243°, $[\alpha]_D^{20}$ +36·6°) and its acetate (m.p. 172–173°), respectively (m.m.p., IR, TLC).

Physical constants of II. m.p. 234–235°, $[\alpha]_D^{20}$ +81·7° in MeOH (*c* 1·90), $[M]_D$ +435° ($[M]_{D,II} - [M]_{D,III}$ +27°, Me-α-D-digitalopyranoside +240°, Me-β-D-digitalopyranoside −100°), UV: λ_{max}^{EtOH} 267 nm (ϵ 14 400). (Found: C, 67·8; H, 8·0. $C_{30}H_{42}O_8$ requires: C, 67·9, H, 8·0%). NMR: δ (in $CDCl)_3$ 1·03 (3H, *s*), 1·21 (3H, *s*), 1·35 (3H, *d*), 2·58 (2H, *d*), 3·51 (3H, *s*), 3·80 (1H, *m*), 4·05 (1H, *m*), 4·26 (1H, *d*, 6Hz, anomeric proton of D-digitalose), 4·95 (2H, *s*), 5·92 (1H, *s*), 6 08 (1H, *m*). Intense yellow fluorescence was observed with $SbCl_3$ reagent. *II-Acetate.* m.p. 173–175°, $[\alpha]_D^{20}$ +48° in $CHCl_3$ (*c* 0·55). (Found: C, 65·1; H, 7·6. $C_{34}H_{46}O_{10}.H_2O$ requires: C, 64 7; H, 7·4%). IR: ν_{max}^{KBr} cm^{-1} 2930, 2840, 1790, 1755, 1630. No absorption for –OH was found between 4000 and 3000 cm^{-1}.

Hydrolysis of II. II (20 mg) was refluxed with 5 ml of 0·05 N HCl–50% MeOH for 30 min, and aglycone and sugar were examined in the usual manner. Although the aglycone was not crystallized, it was identified as IV by direct comparison with authentic sample: UV spectrum (λ_{max}^{MeOH} 385 nm), TLC, and GLC (as trimethylsilate) [relative retention time to adynerigenin (R_t 19·8 min)]: I·03; III: 0·96; IV: 1·03; V: 0·86. Sugar was regarded as D-digitalose (R_t 0 35; specimen L-oleandrose: 0·68, D-diginose: 0·66, D-digitalose: 0·35).

Acknowledgements—We are greatly indebted to Professor T. Reichstein, Univ., Basel, for kind supply of authentic samples of odorosid-*H* and odorosid-*A*. We also thank Professor I. Nishioka, Kyushu Univ., for supply of leaves and Misses M. Kojima and K. Nagase, students of this Univ., for technical assistance. Elementary analyses and measurement of MS were carried out at Hisamitsu Pharmaceutical Co. Ltd., to the members of which our thanks are due.