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QUANTITATIVE VARIATIONS IN THE CARDIAC GLYCOSIDES OF OLEANDER*

TATSUO YAMAUCHI†, FUMIKO ABE, YOSHISHIGE TACHIBANA‡, CHAND K. ATAL§, BRIJ M. SHARMA§ and ZELIHA IMRE||

Faculty of Pharmaceutical Sciences, Fukuoka University, Nanakuma, Jonan-ku, Fukuoka 814-01, Japan; ‡Botanical Garden of Osaka City University, Katano, Osaka 576, Japan; §Regional Research Laboratory, Jammu, India; ||Faculty of Pharmacy, University of Istanbul, Beyazit, Turkey

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Key Word Index—*Nerium odorum*; *N. oleander*; Apocynaceae; oleander; cardenolide; cardiac glycoside; oleandrin; adynerin; oleaside A; oleaside E; chemotaxonomy.

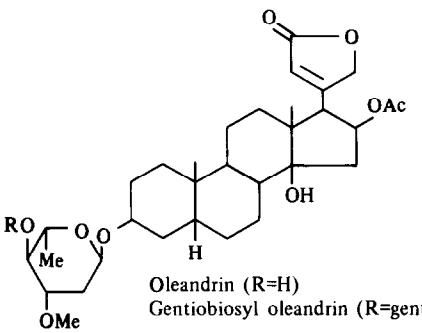
Abstract—Cardiac glycosides of the leaves from 20 horticultural and wild strains of *Nerium* were examined quantitatively. The plants investigated fall into two groups based on their contents of adynerin and oleaside A, or gentiobiosyl adynerin and oleaside E. A large variation in oleandrin content was noted in all samples.

INTRODUCTION

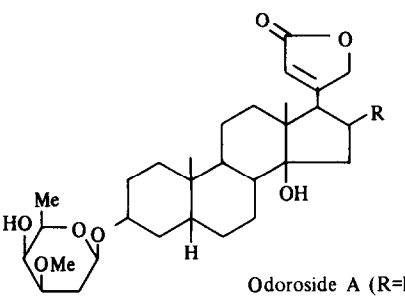
Many cardiac glycosides including oleandrin have been isolated from the leaves of oleander, *Nerium odorum* Sol. (= *N. indicum* Mill.) and *N. oleander* L. Among them, adynerin, an 8,14-epoxycardenolide glycoside, is known as a characteristic companion glycoside of oleander leaves [1]. Recently, we isolated, from the leaves of several horticultural strains of oleander, oleasides A–E [2], inactive glycosides with novel structures which are biogenetically considered to be derived from gentiobiosyl adynerin. In order to verify the distribution of oleaside A and adynerin, or their gentiobiosides, we examined the major cardenolide glycosides including oleandrin, adynerin, and oleaside A, or their gentiobiosides in 20 strains of horticultural or wild oleanders.

*Part 11 in the series "*Nerium*" by T. Y. For Part 10, see ref. [2].

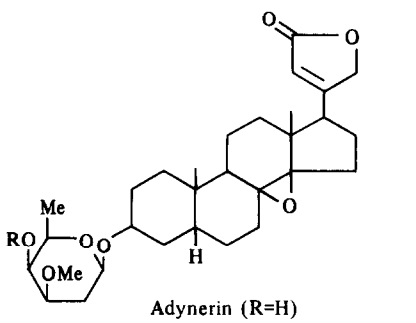
†To whom correspondence should be addressed.



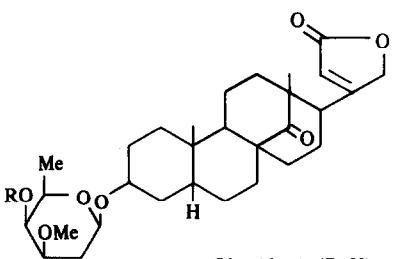
Oleandrin (R=H)
Gentiobiosyl oleandrin (R=gentiobiose)



Odoroside A (R=H)
Nerigoside (R=OAc)



Adynerin (R=H)
Gentiobiosyl adynerin (R=gentiobiose)



Oleaside A (R=H)
Oleaside E (R=gentiobiose)

RESULTS AND DISCUSSION

The plants used in this investigation are listed in Table 1. Some of the horticultural strains were introduced from European botanical gardens as seeds and have been cultivated at the Botanical Garden of Osaka City University. A wild strain of Pakistan (K-28) was collected as seeds and has been cultivated at the Medicinal Plant Garden of Nippon Shinyaku. The leaves of the other wild strains were collected at Foça (T-1), Kasaba (T-2) and Malderesi (T-3) in Turkey, Lindos (G-1) and Kamirros (G-2) of Rhodes in Greece, and Nandani (I-1) and Kud (I-2) in India.

As previously described [3], the native cardenolide glycosides of leaves (triosides and biosides) can be hydrolysed to the corresponding monosides by drying fresh leaves in the oven at 80°; during drying, the original glycosides lose glucose or gentiobiose. Oven-dried leaves of individual strains were percolated with methanol and the extracts were fractionated by means of CC and prep. TLC to yield monosides. The latter were crystallized, and the amounts of the glycosides thus obtained are shown in Table 2.

In air-dried leaves, on the other hand, the original

Table 1. Characteristics and sources of *Nerium* strains

Strains	Characteristics of flower	Sources
Cultivated in Japan		
N-26	Double, pink	The most common strain of *N. odorum* in Japan
N-17	Semidouble, light yellow	Purchased from Hiroshima Seed Co. (1973)
N-19	Single, crimson red	Purchased from Sakata Seed Co. (1965)
N-25	Single, white	Origin unknown
K-5	Semidouble, whitish pink	Jardin E. Museu Lisbon, Portugal (1958)
K-6	Single, whitish pink	Chelsea Physic Garden, Italy (1960)
K-10	Single, cardinal red	California, U.S.A.
K-12	Single, pure white	Origin unknown
K-31	Single, light yellow	Orto Botanico, Hanbury, Italy (1958)
K-107	Single, light crimson	Ariana Agronomique, Tunisia (1958)
K-109	Single, light pink	Jardin Botaniques, Jalta, U.S.S.R. (1960)
K-126	Single, pale white	Jardin Botaniques, Jalta, U.S.S.R. (1960)
K-28	Single, light pink	Pakistan (1964), seeds collected in the field
Leaves collected in the field		
T-1	Single, light pink	Foça, Turkey (1980.1)*
T-2	Single, light pink	Kasaba, Turkey (1980.4)*
T-3	Single, light pink	Malderesi, Turkey (1980.6)*
G-1	Single, light pink	Lindos/Rhodes, Greece (1979.9)*
G-2	Single, light pink	Kamirros/Rhodes, Greece (1979.9)*
I-1	Single, light pink	Nandani, India (1979.9)*
I-2	Single, light pink	Kud, India (1979.11)*

*Date of harvest.

Table 2. Yields of cardenolide monoglycosides (%)* from oven-dried (80°) leaves of *Nerium* strains

Strains	External characteristics†	Oleandrin	Adynerin	Δ^{16}-Adynerin	Oleaside A	Odoroside A	Nerigoside
N-26	od	0.180	0.035	0.032	+	0.003	0.004
K-6	od	0.007	0.073	0.100	+	0.033	0.010
K-12	od	0.180	0.057	0.025	0.004	0.022	0.052
K-28	od	0.370	0.050	0.079	±	0.054	0.051
N-25	md	0.239	0.031	0.048	0.005	0.001	0.010
K-5	ol	0.070	0.033	+	0.072	0.004	0.005
K-31	md	0.141	0.024	0.013	0.023	0.015	0.007
K-10	md	0.067	+	0.017	0.117	0.009	0.015
N-17	ol	0.254	0.001	0.020	0.062	0.008	0.022
N-19‡	ol	0.139	0.002	+	0.069	0.001	+
K-107	ol	0.022	0.008	0.047	0.091	0.042	0.226
K-109	ol	0.018	0.087	0.039	±	0.112	0.126
K-126	ol	0.425	+	+	0.053	0.006	0.003

*+, Detectable, but in very small amount. The underlined values indicate the major monoside in each strain.

†od and ol, Strains showing the external characteristics of *N. odorum* and *N. oleander*; md, those with intermediate characteristics.

‡Yield of neridienone A: 0.047%.

glycosides are retained as gentiobiosyl oleandrin, gentiobiosyl adynerin [4] and oleaside E [2]. In the case of field-collected strains, the leaves were air-dried at room temp. and were percolated with methanol. The methanol extract was then subjected to HPLC (Table 3).

The amount of gentiobiosyl oleandrin in N-26, gentiobiosyl adynerin in K-28, and oleaside E in N-19 were calculated (Table 3) on the bases of the amounts of oleandrin, adynerin and oleaside A in the oven-dried leaves, and HPLC peaks from these three strains were used for calibration. The amounts of the three glycosides in the field-collected strains were obtained by comparison of their HPLC peaks with those of N-26, K-28 and N-19.

Comparison of the amounts of adynerin and oleaside A in the oven-dried leaves

The plants examined can be classified into two groups with respect to the amounts of adynerin and oleaside A; the adynerin group contains adynerin in much larger amounts in comparison with oleaside A (e.g. N-26, N-25, K-6, K-12, K-109 and K-28), and the oleaside group contains oleaside A in larger amounts than adynerin (N-17, N-19, K-10, K-107 and K-126). The few intermediate strains contain adynerin and oleaside A in the ratio of 33:72 (K-5) or 24:23 (K-31).

Comparison of the amounts of gentiobiosyl adynerin and oleaside E in the air-dried leaves

Most of the oleander collected in Pakistan, Turkey, Greece and India were found to belong to the adynerin group, whereas one Indian strain collected in a small valley at Nandani near Jammu, apparently belongs to the oleaside group.

Chemical conversion of adynerigenin into oleagenin was previously established [2], and some of the oleander plants seem to have the biosynthetic enzyme system catalysing this reaction.

Table 3. Yields of cardenolide triosides (%) from shadow-dried leaves of *Nerium* strains

Strains	Gentiobiosyl oleandrin	Gentiobiosyl adynerin	Oleaside E
K-28	0.603	0.082*	—
N-26	0.266*	0.065	0.009
N-19	0.222	0.003	0.112*
T-1†	0.639	0.052	±
T-2‡	0.610	0.041	±
T-3§	0.228	0.046	±
G-1	0.497	0.046	—
G-2	0.300	0.046	—
I-1	0.252	0.004	0.113
I-2	0.379	0.060	—

*The values were calculated on the bases of the yields from the oven-dried leaves.

†Mean values of eight samples.

‡Mean values of four samples.

§Mean values of six samples.

Oleandrin

As shown in Tables 2 and 3, a remarkable variation in oleandrin or gentiobiosyl oleandrin content was observed; the yield of oleandrin varied between 0.42% in K-126 and 0.007% in K-6. N-26, the most popular horticultural strain in Japan, contains 0.18% oleandrin, which is an average figure. A similar variation in gentiobiosyl oleandrin was also observed in the shade-dried samples.

Other constituents

Odoroside A and nerigoside are minor glycosides in the leaves of most plants. K-107 and K-109, however, contain them in larger amounts than other glycosides. In K-5, K-6 and K-10, inactive glycosides, such as adynerin, Δ^{16}-adynerin or oleaside A, are present as major glycosides, and neridienone A [5] was isolated in a yield of 0.047% from the leaves of K-5.

The genus *Nerium* is usually assumed to comprise two species, *N. odorum* and *N. oleander*. The former is indigeneous to India and Pakistan, while the latter grows in the Mediterranean area. The two species are considered to differ in flower fragrance, shape of flower-tube appendages, calyx lobe (erect or spread) and leaf. In this investigation, no correlation was observed between morphology and chemistry. Most of the oleaside type plants among our horticultural strains show the external characteristics of *N. oleander* (Table 2). Oleander collected at Nandani (I-1) represents *N. indicum*, but belongs to the oleaside group also. Oleanders collected in Turkey, Greece and Pakistan belong to the adynerin type (Table 3). The amount of oleandrin in the horticultural strains shows remarkable variation, indicating the possibility of breeding for high or low toxicity.

EXPERIMENTAL

Plant material. The plants used in this study are preserved as specimens of the strains with numbering of series 'K' in the Botanical Garden of Osaka City University and 'N' in the medicinal plant garden of Fukuoka University. The voucher specimens are preserved in the herbarium of the Department of Pharmacognosy, Fukuoka University.

The leaves of each cultivated plant were collected in the flowering season during July–September of 1977 and 1978, and dried in the oven at 80°, immediately after the harvest. The leaves of the wild oleander for the HPLC-method were collected during 1979 and 1980 in the following manner and air-dried at room temp. in the shade; a leaf was collected from every whorl with three leaves at the middle position of all branches, from the top to the ground of 1–3 stems. The HPLC pattern of the glycosides in the samples obtained in this manner were proved to be almost identical with those from 100 g of dried leaves collected at random from the same plant.

Gravimetric method. The dried powdered leaves (330–600 g) were percolated with 5 l. MeOH. The percolate was concd *in vacuo* to 500 ml and an equal vol. of H_2O was added. The mixture was filtered and the filtrate was extracted with C_6H_6 (600 ml × 3). The C_6H_6 was evaporated *in vacuo* and the residue was crystallized first from MeOH to give adynerin (or oleandrin, in some cases). The mother liquor fraction was then crystallized from EtOAc–hexane to give oleandrin (or oleaside A, in some cases). The mother liquor fraction was subjected to CC on Si gel (C_6H_6–Me_2CO) and prep. TLC (EtOAc–hexane) giving additional amounts of the above-mentioned three glycosides together with Δ^{16}-adynerin, nerigoside and odoroside A. All

glycosides were crystallized from suitable solvents and yields are listed in Table 2.

HPLC method. The leaves, dried in the shade at room temp. (15–20 g), were powdered and 1 g of them was percolated with 30 ml of MeOH. The percolate was then concd *in vacuo* to dryness. The residue was dissolved in 2 ml 50% MeCN, and a 1 ml aliquot was passed through Sep-Pak C18. An additional 2 ml 50% MeCN was also passed through the Sep-Pak. The whole effluent was combined together and 5 μl aliquot of the effluent was subjected to HPLC (Waters ALC/GPC 200, equipped with Radial Pak C18). The UV-detector was adjusted to 220 nm. As a solvent system, 27.5% MeCN was used (3.5 ml/min). R_t (min): gentiobiosyl oleandrin, 6.33; oleaside E, 8.54; gentiobiosyl adynerin, 12.79.

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