CHEMISTRY LETTERS, pp. 393-396, 1980.

THREE NEW DAPHNIPHYLLUM ALKALOIDS WITH AN $\alpha\beta,\gamma\delta$-UNSATURATED ESTER GROUP FROM DAPHNIPHYLLUM GRACILE GAGE

Shosuke YAMAMURA,* J.A. LAMBERTON,† Masatake NIWA, Keiko ENDO, and Yoshimasa HIRATA

Faculty of Pharmacy, Meijo University, Tempaku-ku, Nagoya 468

† Division of Applied Chemistry, C.S.I.R.O., Melbourne 3001, Australia

Three new alkaloids have been isolated from the bark of Daphniphyllum gracile Gage, and their structures with an $\alpha\beta,\gamma\delta$-unsaturated ester group also been elucidated on the basis of their spectral and chemical properties. In addition, it is chemotaxonomically important that daphniphylline, one of the main alkaloids found in the Japanese species, has also been detected in this experiment, while this alkaloid has not been found in the leaves of the same plant.

Recently, a disease with jaundice, colic and photophobia as main symptoms broke out among grazing cattle in Hokkaido,[1] and it was demonstrated by Sonoda and his co-workers to be a plant poisoning caused by Daphniphyllum humile Maxim.,[1] which contained many alkaloids affecting the liver specifically.[2] In connection with such toxicological as well as chemotaxonomical aspects, we further examined alkaloidal components of the bark sample of Daphniphyllum gracile Gage growing in New Guinea,[3] and could isolate three new alkaloids (daphgracine, daphgraciline and hydroxydaphgraciline). In the present paper, we wish to describe the isolation and structures of these alkaloids, and their biogenesis is also presented.

The bark of Daphniphyllum gracile Gage growing in the Trust Territory of New Guinea was collected by one of us (J.A.L.) late in January. According to the same procedure as described in the previous paper,[4] the air-dried bark of the plant was extracted with MeOH at 40 °C, and then the extracts were treated as usual to afford crude alkaloids as a dark brown oil (ca. 0.16% yield), which was chromatographed on alumina (Nakarai Chemical Co., Ltd., ca. 300 mesh) using $CHCl_3$ to give a mixture of several compounds. This mixture was rechromatographed on alumina and eluted with benzene to afford daphniphylline[5] in 0.0001% overall yield. Further elution with ether gave a mixture of three alkaloids, which was separated by preparative TLC [Kieselgel PF_{245}; hexane-Et_2O-Et_2NH (10 : 10 : 1)] to give daphnigraciline (1),[4] daphgracine (2) and daphgraciline (3) in 0.00009, 0.00009 and 0.0003% overall yields, respectively. Further elution with EtOAc followed by preparative

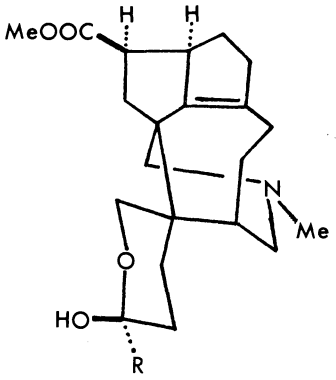
TLC [Kieselgel PF_{254}; hexane-Et_2O-Et_2NH (5 : 10 : 1)] afforded hydroxydaphgraciline (4) in 0.00008% overall yield.

Of these alkaloids, the structures of both daphniphylline[5] and daphnigraciline (1)[4] have already been elucidated. From a chemotaxonomical aspect, it is noted that the former is included in the bark of Daphniphyllum gracile Gage, although it has not been detected in the leaves of the same plant. The physical data of the remaining new alkaloids are shown below.

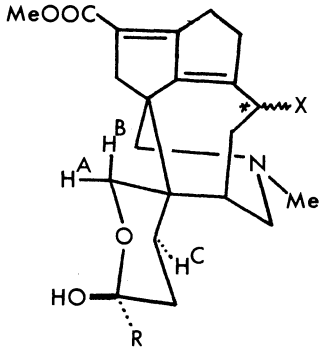
Daphgracine (2) as a colorless viscous liquid: $C_{24}H_{35}O_4N$; m/e 401(M^+), 383 and 370; ν_{max}(film) 3450, 1690, 1655 and 1620 cm^{-1}; λ_{max}(MeOH) 298 nm (ε, 7000); δ(pyridine-d_5) 1.11(3H, d, J= 6Hz), 1.17(3H, d, J= 6Hz), 2.13(3H, s), 3.67(3H, s), 3.82(1H, br.d, J= 12Hz) and 4.21(1H, d, J= 12Hz).

Daphgraciline (3) as a colorless viscous liquid: $C_{23}H_{33}O_4N$; m/e 387(M^+), 369 and 356; ν_{max}(film) 3450, 1690, 1655 and 1620 cm^{-1}; λ_{max}(MeOH) 298 nm (ε, 9000); δ(pyridine-d_5) 1.12(3H, t, J= 7Hz), 2.16(3H, s), 3.70(3H, s), 3.90(1H, br.d, J= 12Hz) and 4.32(1H, d, J= 12Hz); δ($CDCl_3$) 7.68(q), 21.6 (t), 25.8(t), 26.1(t), 27.4(t), 27.8(t),32.8(d), 35.6(t), 35.9(s), 42.0(t), 42.9(t), 46.0(q), 46.3 (s), 50.7(q), 55.8(t), 61.6(t), 62.5(t), 96.0(s), 115.7(s), 149.4(s), 153.5(s), 166.6(s) and 168.7(s).

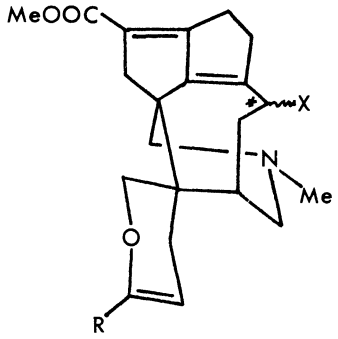
Hydroxydaphgraciline (4) as a colorless viscous liquid: $C_{23}H_{33}O_5N$; m/e 403(M^+), 385, 374, 372, 367, 356 and 354; ν_{max}(film) 3400br., 1695, 1660 and 1625 cm^{-1}; λ_{max}(MeOH) 294 nm (ε, 7000); δ(pyridine-d_5) 1.12(3H, t, J= 7Hz), 2.12(3H, s), 3.67(1H, br.d, J= 12Hz), 3.68(3H, s), 4.23(1H, superimposed on the doublet at δ4.29) and 4.29(1H, d, J= 12Hz).



1 R = Et
5 R = i-Pr



2 R = i-Pr, X = H
3 R = Et, X = H
4 R = Et, X = OH



6 R = Et, X = H
7 R = Et, X = OAc

As seen in the case of daphnigracine (5) and daphnigraciline (1),[4] the spectral data of both 2 and 3 indicate that these two alkaloids must be quite similar to each other except for the following point: the PMR spectrum of 2 has two doublets at δ1.11 and 1.17 assignable to an iso-

propyl group, while one methyl triplet is observed at δ1.12 in the case of the latter. Furthermore, the structures of these alkaloids including the stereochemistry could be elucidated on the basis of an exhaustive comparison of the IR, UV and CMR spectra between 1 and 3.

The IR and UV spectra of 3 indicate the presence of an $\alpha,\beta,\gamma,\delta$-unsaturated ester group (ν_{max} 1690 cm^{-1}; λ_{max} 298 nm), whereas daphnigraciline (1) has no conjugated ester group (ν_{max} 1730 cm^{-1}). In the CMR spectrum of 3, there are five signals at δ115.7, 149.4, 153.5, 166.6 and 168.7 in lower magnetic field, the four of which can be due to olefinic carbon atoms. The remaining one is due to the ester CO group. In the case of 1, there are only two olefinic carbon atoms in addition to one ester CO group.

From these data, the stereostructure of daphgraciline can be represented by 3, in which the configuration of the OH group is based on the PMR spectrum: a sharp doublet at δ4.32 due to the geminal proton (H^A), which is in a 1,3-diaxial relationship to the OH group, is observed at the magnetic field lower than the PMR signal at δ3.90 assignable to the equatorial geminal proton (H^B) which is coupled with a proton (H^C) along the "W" path. On treatment with Ac_2O-AcOH (1 : 1) at room temperature, furthermore, daphgraciline (3) was converted into the corresponding dehydration product (6), whose structure was confirmed by its spectral data [6: $C_{23}H_{31}O_3N$; m/e 369(M^+); $\delta(CDCl_3)$ 1.10(3H, t, J= 7Hz), 2.14(3H, s), 3.47(1H, d, J= 11Hz), 3.69(3H, s), 4.07(1H, dd, J= 11, 2Hz) and 4.35(1H, m); $\delta(CDCl_3)$ 11.5(q), 25.8(t), 26.1(t), 26.5(t), 27.7(t), 27.9(t), 34.3(d), 35.7(s), 42.0(t), 42.9(t), 46.0(q), 46.3(s), 50.7(q), 56.3(t), 61.9(t), 69.0(t), 92.1(d), 115.5(s) 150.0(s), 153.4(s), 155.3(s), 166.6(s) and 168.8(s)].

The stereostructure of daphgracine must be also represented by 2, as judged from its PMR signals at δ3.82 and 4.21.

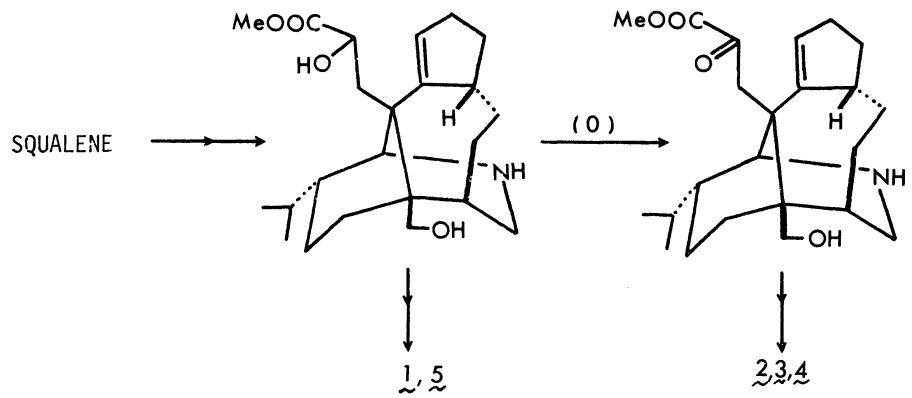
The remaining new alkaloid, hydroxydaphgraciline (4), has the same carbon skeleton as that of 3, as judged from its spectral data. In addition, it has one secondary OH group (δ4.23) which is shifted to δ5.52(1H, br.d, J= 5Hz) on treatment with Ac_2O-AcOH (1 : 1) giving the corresponding dehydration monoacetate (7).[6] Furthermore, both chemical shift (δ5.52) and coupling constant (J= 5Hz) strongly suggest that the secondary OH group in 4 is located at the asterisk-position.[7]

These alkaloids (2, 3 and 4) may be produced from squalene,[8] as shown in Scheme 1. Extensive studies on their toxic activities are in progress.

References and footnotes

1. M. Sonoda, M. Tasaka, K. Takahashi, M. Koiwa, S. Minami, M. Iwase, and K. Yashiro, J. Japan Veterinary Medical Association, 31, 140 (1978).

2. S. Yamamura and Y. Terada, Chem. Lett., 1976, 1318.



Scheme 1. Biogenesis of daphnigraciline, daphgracine and related alkaloids

3. T.G. Hartley, E.A. Dunstone, J.S. Fitzgerald, S.R. Johns, and J.A. Lamberton, Lloydia, 36, 217 (1973).

4. S. Yamamura, J.A. Lamberton, H. Irikawa, Y. Okumura, M. Toda, and Y. Hirata, Bull. Chem. Soc. Jpn., 50, 1836 (1977).

5. H. Irikawa, N. Sakabe, S. Yamamura, and Y. Hirata, Tetrahedron, 24, 5691 (1968).

6. Although the molecular ion peak did not appear in the mass spectrum of 7, its structure was supported by its IR and PMR spectra: ν_{max}(film) 1725, 1695sh., 1660sh. and 1625 cm^{-1}; $\delta(CDCl_3)$ 0.98(3H, t, J= 7Hz), 2.06(3H, s), 2.20(3H, s), 3.50(1H, d, J= 11Hz), 3.82(1H, dd, J= 11,2Hz), 4.36(1H, m) and 5.52(1H, br.d, J= 5Hz).

7. The configuration of the OH group has not been determined on the basis of the coupling constant (J= 5Hz), because the seven-membered ring including the OH group can adopt two possible conformations.

8. K.T. Suzuki, S. Okuda, H. Niwa, M. Toda, Y. Hirata, and S. Yamamura, Tetrahedron Lett., 1973, 799.

(Received February 15, 1980)