[Chem. Pharm. Bull. 19(8)1747—1749(1971)]

UDC 547.991.02 : 581.192 : 582.752.1

# The Structure of Nigakilactone J

The structures of a number of bitter principles isolated from *Picrasma ailanthoides* PLANCHON (=*P. quassioides* BENNETT; Simaroubaceae) have been reported.[1-7] In addition to the previously described substances,[1,3,5,6] we have recently isolated a new bitter principle, nigakilactone J. The evidence establishing structure Ia for nigakilactone J is reported in the present communication.

The molecular formula of $C_{23}H_{34}O_7$ ($M^+$ at *m/e* 422) was given for nigakilactone J, mp 240—241°, $[\alpha]_D$ +42° (EtOH). The infrared (IR) ($\nu_{max}$ 3550, 3450, 1735, 1722 sh, 1704 and 1250 $cm^{-1}$), proton magnetic resonance (PMR) ($\delta$ 1.93 ppm and $\delta$ 4.22 ppm; Table I) and the mass [*m/e* 362, $(M\text{-}AcOH)^+$] spectra suggest the presence of a $\delta$-lactone, an acetoxyl and a saturated ketone function, together with that of the hydroxyl group. The ultraviolet (UV) spectrum shows no absorption maximum due to a conjugated system. The presence of a methoxyl group, two secondary and two tertiary methyls is shown in the PMR spectrum (Table I). No signal due to an olefinic proton is observed.

TABLE I. PMR Spectral Data ($\delta$ in ppm)[a]

| Compounds | *sec*-$CH_3$ | *tert*-$CH_3$ | -O-CO-$CH_3$ | CH-$OCH_3$ | -$OCH_3$ | C-CH-O⁻ (7) | CH-OH | CH-OAc |
|---|---|---|---|---|---|---|---|---|
| Ia | 0.90 d $J$=6 | 1.26 s | 1.93 s | 3.18 q $J$=9 $J$=11 | 3.45 s | 4.22m | 4.74m | 5.28 q $J$=12 $J$=9 |
| | 1.04 d $J$=7 | 1.28 s | | | | | | |
| Ib | 0.96 d $J$=6 | 1.27 s | 2.02 s | 3.11 q $J$=9 $J$=11 | 3.40 s | 4.16m | — | 5.28 q $J$=12 $J$=9 |
| | 1.03 d $J$=7 | 1.32 s | 2.11 s | | | | | 5.58 q[b] $J\fallingdotseq$13 $J\fallingdotseq$7 |
| IIIc | 1.01 d $J$=6 | 1.27 s | 1.95 s | 3.20 q $J$=9 $J$=11 | 3.42 s | 4.14m | — | 5.22 q $J$=11 $J$=9 |
| | 1.06 d $J$=7 | 1.27 s | | | 3.54 s | | | |
| IVa | 0.92 d $J$=6 | 1.22 s | — | | 3.58 s | 4.11m | 4.72m | — |
| | 1.04 d $J$=6 | 1.40 s | | | | | | |

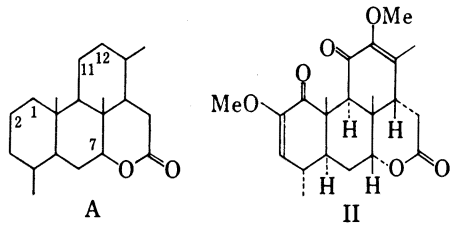
a) Determined in $CDCl_3$ at 60 MHz. b) signal due to $C_{(9)}$-H; the others due to $C_{(11)}$-H

Acetylation of nigakilactone J gave its monoacetate (Ib), $C_{25}H_{36}O_8$ ($M^+$ at *m/e* 464), mp 275—276° (PMR: Table I), which shows no IR absorption due to hydroxyl group. Therefore, one hydroxyl group should be present in nigakilactone J.

The nature of seven oxygen atoms involved in nigakilactone J is thus characterized. The above spectral data along with the molecular formula of nigakilactone J are best inter-

1) T. Murae, T. Tsuyuki, T. Nishihama, S. Masuda and T. Takahashi, *Tetrahedron Letters*, **1969**, 3013.
2) H. Hikino, T. Ohta and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **18**, 219 (1970).
3) T. Murae, T. Ikeda, T. Tsuyuki, T. Nishihama and T. Takahashi, *Bull. Chem. Soc. Japan*, **43**, 969 (1970).
4) H. Hikino, T. Ohta and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **18**, 1082 (1970).
5) T. Murae, T. Ikeda, T. Tsuyuki, T. Nishihama and T. Takahashi, *Bull. Chem. Soc. Japan*, **43**, 3021 (1970).
6) T. Murae, T. Ikeda, T. Tsuyuki, T. Nishihama and T. Takahashi, *Chem. Pharm. Bull.* (Tokyo), **18**, 2590 (1970).
7) H. Hikino, T. Ohta and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **19**, 212 (1971).

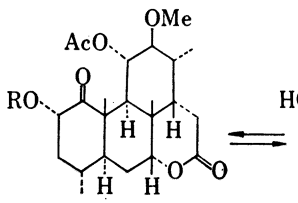
preted on the basis of the *saturated* skeletal structure (A) related to quassin (II).[8] In the PMR spectrum of nigakilactone J, there appear a quartet (1H, $\delta$ 5.28 ppm, $J$=12 and 9 Hz; CH-OAc) and a quartet (1H, $\delta$ 3.18 ppm, $J$=9 and 11 Hz; CH-OMe), whose signal patterns are closely similar to those of nigakilactone C (IIIc)[1] (Table I). This leads to the location of an acetoxyl group on $C_{11}$ and a methoxyl group on $C_{12}$ for nigakilactone J as that for nigakilactone C (IIIc). All bitter substances hitherto isolated[1-7] from the same plant has a carbonyl and a methoxyl (or a hydroxyl) group at $C_1$ and $C_2$, respectively. With this information, the location of a carbonyl group on $C_1$ and of a hydroxyl group on $C_2$ is suggested for nigakilactone J. Thus, nigakilactone J could be formulated as Ia. This was confirmed by the following transformations.

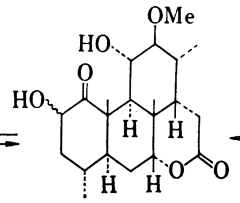


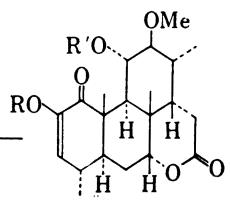
A



II







Ia : R=H
Ib : R=Ac

IVa : 2$\alpha$-OH
IVb : 2$\beta$-OH

IIIa : R=$CH_3$, R'=H
IIIb : R=H, R'=H
IIIc : R=$CH_3$, R'=Ac

Treatment of known nigakilactone B (IIIa)[1] with hydrochloric acid in acetic acid gave nornigakilactone B (IIIb), $C_{21}H_{30}O_6$ ($M^+$ at $m/e$ 378). The presence in IIIb of a diosphenol moiety is shown by the shift of UV maximum in ethanol from 280 nm to 335 nm on addition of alkali.

Reduction of nornigakilactone B (IIIb) in acetic acid under reflux with zinc yielded a mixture of the dihydro derivatives (epimers at $C_2$; IVa and IVb), which was separated by preparative thin-layer chromatography to afford IVa (major product), $C_{21}H_{32}O_6$ ($M^+$ at $m/e$ 380), mp 210—211° (Table I), and IVb (minor product), $C_{21}H_{32}O_6$ ($M^+$ at $m/e$ 380), mp 231—232.5°. The same mixture (IVa and IVb) was obtained by treatment of Ia with hydrochloric acid in acetic acid. The absence of the UV maximum due to a conjugated system is shown for IVa. The mass spectra of IVa and IVb are essentially identical, although their IR spectra are different.

Acetylation of the major product (IVa) gave an acetyl derivative which was shown to be identical with Ib. The structure of nigakilactone J is thus represented by Ia.

The PMR signal due to a proton on $C_2$ of Ib (Table I) appears at $\delta$ 5.58 ppm as a quartet. The observed coupling constants ($J$≒13 and 7 Hz) suggest that this proton is in axial conformation (2$\beta$-H configuration). This leads to the stereochemistry (Ia) for nigakilactone J.

8) Z. Valenta, S. Papadopoulos and C. Podešva, *Tetrahedron*, **15**, 100 (1961); Z. Valenta, A. H. Gray, D.E. Orr, S. Papadopoulos and C. Podešva, *Tetrahedron*, **18**, 1433 (1962).

*Department of Chemistry,*
*Faculty of Science,*
*The University of Tokyo*
*Bunkyo-ku, Tokyo*

TATSUSHI MURAE
TAKAHIKO TSUYUKI
TAKEYOSHI TAKAHASHI

Received May 29, 1971

[Chem. Pharm. Bull. 19(8)1749—1750(1971)]

*UDC 615.31.011.4.076.9*

## Substrate Induced Difference Spectrum of Microsomal P-450 in Human Liver

Substrate-P-450 difference spectra, which are induced by addition of various substrates into suspension of liver microsomes, have been reported to classify into two or three groups. In rat liver microsomes, hexobarbital, aminopyrine and SKF525-A cause type I difference spectrum and aniline causes type II difference spectrum.[1-3]

Schenkman, *et al.*[4] and Kitagawa, *et al.*[5] have reported that hexobarbital shows similar type II difference spectrum when it is added into microsomes treated with 3-methylcholanthrene.

Additionally, the author, *et al.*[6] has reported that hexobarbital shows similar type I spectrum when low concentrations of the substrate are used and similar type II spectrum when rather high concentrations of the substrate are employed in 3-methylcholanthrene treated microsomes.

Studies on some activities of drug-metabolizing enzymes in human liver have been realized by Kuntzman, *et al.*,[7] Creaven, *et al.*,[8,9] and the authors.[10]

In the present paper, we wish to report substrate induced P-450 difference spectra in human liver microsomes.

Adult human livers which were isolated in judicial or administrative dissection within 24 hr after death were used, and they were from individuals without any history of drug poisoning or liver illness. About 50 g of the livers were sliced and washed more than three times with ice-cold 1.15% KCl solution to remove blood. The slices were homogenized with 2 volumes of ice-cold 1.15% KCl solution. The homogenate was centrifuged at −2.0°—2.0°, 9000×*g* for 20 min. The supernatant was recentrifuged at 105000×*g* for 1 hr, and the microsomal pellet was suspended in 0.1 M phosphate buffer pH 7.5. The suspension was used for measurements of P-450 content and P-450-substrate difference spectra.

P-450 content was measured by the method according to Omura, *et al.*[11] Microsomal protein was determined according to the method of Lowry, *et al.*[12]

1) H. Remmer, J.B. Schenkman, R.W. Estabrook, H. Sasame, J. R. Gillette, D.Y. Cooper, S. Narasimhulu, and O. Rosenthal, *Mol. Pharmacol.*, **2**, 187 (1966).
2) J.B. Schenkman, H. Remmer, and R.W. Estabrook, *Mol. Pharmacol.*, **3**, 113 (1967).
3) Y. Imai and R. Sato, *Biochem. Biophys. Res. Commun.*, **22**, 620 (1966).
4) J.B. Schenkman, H. Grein, M. Zange, and H. Remmer, *Biochem. Biophys. Acta*, **171**, 23 (1969).
5) H. Kitagawa, K. Koyama, and T. Kamataki, The 89th Annual Meeting of Pharmaceutical Society of Japan, Nagoya, April, 1969.
6) T. Kamataki, H. Fukazawa, and H. Kitagawa, *Yakugaku Zasshi*, **91**, 142 (1971).
7) R. Kuntzman, L.C. Mark, L. Brand, M. Jacobson, W. Levin, and A.H. Conney, *J. Pharmacol. Exptl. Therap.*, **152**, 151 (1966).
8) P.J. Creaven, D.V. Parke, and R. T. Williams, *Biochem. J.*, **85**, 5 (1962).
9) P.J. Creaven and R. T. Williams, *Biochem. J.*, **87**, 19 (1963).
10) H. Kitagawa and T. Kamataki, *Chem. Pharm. Bull.* (Tokyo), **19**, 827 (1971).
11) T. Omura and R. Sato, *J. Biol. Chem.*, **239**, 2370 (1964).
12) O.H. Lowry, N. J. Rosebrough, A.L. Farr,and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).