Phytochemistry, Vol. 22, No. 12, pp. 2853–2855, 1983.
Printed in Great Britain.

0031–9422/83 $3.00 + 0.00

ISOLATION OF INDIVIDUAL TYPE II CYANOLIPIDS FROM *NEPHELIUM LAPPACEUM**

MUGIO NISHIZAWA, KENJI ADACHI, SETIJATI SASTRAPRADJA† and YUJI HAYASHI

Department of Chemistry, Faculty of Science, Osaka City University, Osaka 558, Japan; †The National Biological Institute, Bogor, Indonesia

(*Revised received* 17 *May* 1983)

Key Word Index—*Nephelium lappaceum*; Sapindaceae; seeds; type II cyanolipids; HPLC purification.

Abstract—Three kinds of type II cyanolipids were isolated as pure forms from *Nephelium lappaceum* using HPLC. The full structures were established by spectral and chemical investigations.

INTRODUCTION

Cyanolipids are a group of plant lipids, classified into four types I–IV [1, 2], which occur in the seed oil of sapindaceous plants. This interesting class of lipids has never been purified completely and structural assignments have been carried out using mixtures. Described herein are the first complete separation and full characterization of the type II cyanolipids obtained from the seeds of an Indonesian fruit, *Nephelium lappaceum*, [3].

RESULTS AND DISCUSSION

Extraction of powdered seeds of *N. lappaceum* with hexane gave a lipid mixture as a pale yellow semi-solid. Column chromatography on silica gel afforded triacylglycerols (25 % dry wt) and the type II cyanolipid (4.5 % dry wt). The latter component showed a sharp absorption band at 2230 cm^{-1} in the IR spectrum and gave a single spot on TLC with a variety of solvent systems. However, HPLC using a Develosil C8-5 column with acetonitrile as eluant revealed inhomogeneity of this lipid, which consisted of at least five components. Preparative HPLC under the same conditions provided three major cyanolipids, N-IIb, N-IIc and N-IId, as pure colourless semi-solids. Elemental analysis indicated the molecular formulae of N-IIb–d to be $C_{41}H_{71}NO_4$, $C_{43}H_{75}NO_4$ and $C_{43}H_{77}NO_4$, respectively. Although each cyanolipid showed entirely superimposable IR spectra (3060, 2230, 1750, 1645 cm^{-1}), they were distinguishable in the olefinic region of their ^{1}H NMR and ^{13}C NMR spectra. The common dihydroxyisobutenyl cyanide moiety of these compounds showed characteristic ^{13}C NMR signals at δ114.8 (*s*, C-1), 98.7 (*d*, C-2), 155.0 (*s*, C-3), 62.8 (*t*, C-4), and 61.7 (*t*, C-5). An incorrect assignment of the nitrile carbon was reported by Mikolajczak *et al.*, who assigned one of the olefinic carbon signals (δ129.7) as that of the nitrile carbon [4]. ^{1}H NMR spectra also supported the presence of this skeleton: δ5.58 (1H, *br*, H-2), 4.90 (2H, *d*, J = 0.8 Hz, H-4), and 4.72 (2H, *d*, J = 1.8 Hz, H-5).

The fatty acid parts of each cyanolipid were characterized by GC/MS analysis of their ozonolysis products (Table 1) in the following manner. Methanolysis of N-IIb provided a single methyl ester with m/z 296 ($[M]^+$ for $C_{17}H_{33}CO_2Me$). Since distinction of the olefinic regioisomer of long-chain unsaturated fatty acids is usually very difficult by spectroscopic methods, this product was successively treated with ozone, sodium borohydride, Jones reagent and diazomethane to give two fragment components, a mono-ester with m/z 172 ($[M]^+$ for $C_8H_{17}CO_2Me$) and a diester with m/z 185 ($[M - OMe]^+$ for $MeO_2C(CH_2)_7CO_2Me$). Thus, the full structure of N-IIb was rigidly established to be **1**. N-IIc provided two kinds of methanolysis products with m/z 296 ($[M]^+$ for $C_{17}H_{33}CO_2Me$) and m/z 324 ($[M]^+$ for $C_{19}H_{37}CO_2Me$). Further degradation of this mixture as described above afforded three kinds of ester fragments with m/z 172 ($[M]^+$ for $C_8H_{17}CO_2Me$), m/z 185 ($[M - OMe]^+$ for $MeO_2C(CH_2)_7CO_2Me$) and m/z 213 ($[M - OMe]^+$ for $MeO_2C(CH_2)_9COMe$). As a result, structure **2** was assigned to N-IIc. N-IId also gave two different methyl esters with m/z 296 ($[M]^+$ for $C_{17}H_{33}CO_2Me$) and 326 ($[M]^+$ for $C_{19}H_{39}CO_2Me$). The former was the same methyl oleate as that obtained from N-IIb, and the latter was a saturated ester, methyl eicosanoate. Thus, structure **3** was assigned to N-IId.

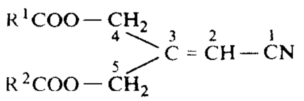
The double-bond geometry of the octadecenoyl and eicosenoyl residues was assigned to be *Z* on the basis of ^{13}C NMR examination of the allylic carbons (δ27.4), since authentic methyl oleate showed the allylic carbon signal at δ27.2 and the corresponding *E*-isomer (methyl elaidate) showed this signal at δ32.6.

EXPERIMENTAL

Isolation of cyanolipids N-IIb (**1**), *N-IIc* (**2**) *and N-IId* (**3**). Dried seeds (750 g) of *N. lappaceum* L., collected in Bogor, Indonesia in February 1982, were powdered using a mechanical mixer and the resulting powder was continuously extracted for 2 days with hexane (1 l.) using a Soxhlet apparatus. Concn of the solvent gave a pale yellow semi-solid (212 g), of which 40 g was subjected to CC on silica gel (350 g) using hexane–EtOAc (10:1 then 4:1) to give a mixture of triacylglycerols (32.1 g, 25 % dry wt) and a mixture of cyanolipids (5.8 g, 4.5 % dry wt).

---

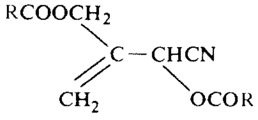
*Dedicated to Emeritus Professor Takeo Sakan on his 70th birthday.



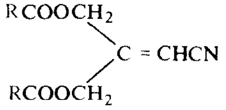
1 $R^1 = Me(CH_2)_7CH\overset{Z}{=}CH(CH_2)_7$; $R^2 = Me(CH_2)_7CH\overset{Z}{=}CH(CH_2)_7$

2 $R^1 = Me(CH_2)_7CH\overset{Z}{=}CH(CH_2)_7$; $R^2 = Me(CH_2)_7CH\overset{Z}{=}CH(CH_2)_9$

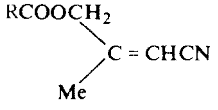
3 $R^1 = Me(CH_2)_7CH\overset{Z}{=}CH(CH_2)_7$; $R^2 = n\text{-}C_{19}H_{39}$



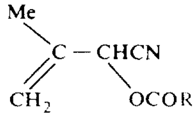
I



II



III



IV

Table 1. GC/MS analysis of cyanolipid degradation products

Cyanolipid	Methanolysis products	Ozonolysis products	
N-IIb	$C_{17}H_{33}CO_2Me$ 296 $[M]^+$	$C_8H_{17}CO_2Me$ 172 $[M]^+$	$MeOCO(CH_2)_7CO_2Me$ 185 $[M-OMe]^+$
N-IIc	$C_{17}H_{33}CO_2Me$ 296 $[M]^+$	$C_8H_{17}CO_2Me$ 172 $[M]^+$	$MeOCO(CH_2)_7CO_2Me$ 185 $[M-OMe]^+$
	$C_{19}H_{37}CO_2Me$ 324 $[M]^+$	$C_8H_{17}CO_2Me$ 172 $[M]^+$	$MeOCO(CH_2)_9CO_2Me$ 213 $[M-OMe]^+$
N-IId	$C_{17}H_{33}CO_2Me$ 296 $[M]^+$	$C_8H_{17}CO_2Me$ 172 $[M]^+$	$MeOCO(CH_2)_7CO_2Me$ 185 $[M-OMe]^+$
	$C_{19}H_{39}CO_2Me$ 326 $[M]^+$		

Prep. HPLC using a Develosil C8-5 column, 4 × 100 mm, and MeCN as eluant at a flow rate of 2 ml/min provided three major cyanolipids (RI detector), N-IIb (**1**), (R_t *ca* 7.5 min), N-IIc (**2**) (R_t *ca* 11 min) and N-IId (**3**) (R_t *ca* 15 min), as colourless semi-solids in a ratio of 10:29:35.

N-IIc (**2**). IR (neat) cm^{-1}: 3060, 2230, 1750, 1645; ^1H NMR ($CDCl_3$): δ0.95 (6H, *t*, $J = 7$ Hz), 1.30 (*br*), 1.68 (4H, *t*, $J = 7$ Hz), 2.08 (8H, *m*), 2.40 (4H, *t*, $J = 7$ Hz), 4.72 (2H, *d*, $J = 1.8$ Hz), 4.90 (2H, *d*, $J = 0.8$ Hz), 5.35 (4H, *t*, $J = 7$ Hz), 5.58 (1H, *m*); ^{13}C NMR ($CDCl_3$): δ14.2 *q*, 22.9 *t*, 25.0 *t*, 27.3 *t*, 32.1 *t*, 33.9 *t*, 34.1 *t*, 61.7 *t*, 62.8 *t*, 98.7 *d*, 114.8 *s*, 129.8 *d*, 130.0 *d*, 130.2 *d*, 155.0 *s*, 172.6 *s*, 173.0 *s*. (Calc. for $C_{43}H_{75}NO_4$: C, 77.08; H, 11.28; N, 2.09. Found: C, 77.03; H, 11.38; N, 2.14%.)

N-IIb (**1**). IR, ^1H NMR and ^{13}C NMR spectra were entirely superimposable with those of **2**. (Calc. for $C_{41}H_{71}NO_4$: C, 76.70; H, 11.15; N, 2.18. Found: C, 76.43; H, 11.31; N, 2.15%.)

N-IId (**3**). IR spectrum was superimposable with that of **2**; ^1H NMR spectrum differed only from that of **2** in the intensity ratio of two signals at δ2.08 (4H, *m*) and 5.35 (2H, *t*, $J = 7$ Hz); ^{13}C NMR spectrum showed two olefinic carbon signals at δ129.8 *d*, and 130.2 *d* due to fatty acid moieties. (Calc. for $C_{43}H_{77}NO_4$: C, 76.84; H, 11.55; N, 2.11. Found: C, 76.91; H, 11.65; N, 2.11%.)

Methanolysis of N-IIb (**1**), *N-IIc* (**2**) *and N-IId* (**3**). A soln of N-IIb (**1**) (11.8 mg, 17 μmol) and LiOH (132 mM MeOH soln 5 μl, 0.66 μmol) in dry MeOH (3 ml) was heated at reflux temp. for 2 days under an Ar atmosphere. The conc. residue was subjected to CC on silica gel (3 g) using petrol–Et_2O (10:1) to give methyl oleate (6.4 mg, 65% yield).

N-IIc (**2**) and N-IId (**3**) were treated as above and the mixture of methyl esters was obtained, respectively.

Degradation of methyl oleate. A MeOH soln of methyl oleate obtained above was treated with O_3 at −78° for 10 min and the resulting soln treated with $NaBH_4$ (5 mg) and warmed to room temp. The syrup obtained by the usual work-up was dissolved in Me_2CO and treated with Jones reagent. To the Et_2O extract of the reaction mixture was added an excess of CH_2N_2–Et_2O and the mixture then concd. This product was then subjected to GC/MS using an OV-1 column.

The methyl ester mixtures obtained from **2** and **3** were treated as above to give samples for GC/MS analysis.

Acknowledgements—We thank The Naito Foundation (1980, 1981) and The Toyota Foundation (1981) for financial aid.

REFERENCES

1. Mikolajczak, K. L. (1977) *Prog. Chem. Fats Other Lipids* **15**, 97.
2. Mikolajczak, K. L., Smith, C. R., Jr. and Tjarks, L. W. (1970) *Lipids* **5**, 672.
3. Mikolajczak, K. L., Smith, C. R., Jr. and Tjarks, L. W. (1970) *Lipids* **5**, 812.
4. Mikolajczak, K. L. and Weisleder, D. (1978) *Lipids* **13**, 514.

Phytochemistry, Vol. 22, No. 12, pp. 2855–2856, 1983.
Printed in Great Britain.

0031-9422/83 $3.00 + 0.00

STRUCTURE OF (+)-RANGIFORMIC ACID*

SIEGFRIED HUNECK and WOLFGANG STEGLICH†

Institute of Plant Biochemistry, Research Centre for Molecular Biology and Medicine of the Academy of Sciences of the GDR, GDR-401 Halle/Saale, Weinberg, German Democratic Republic; †Institute of Organic Chemistry and Biochemistry, University of Bonn, D-5300 Bonn 1, Gerhard-Domagk-Str. 1, Federal Republic of Germany

(*Received* 28 *February* 1983)

Key Word Index—*Cladonia rangiformis*; Cladoniaceae; lichen; (+)-rangiformic acid; (+)-3*S*-methoxycarbonylheptadecane-1,2*S*-dicarboxylic acid.

Abstract—(+)-Rangiformic acid from the lichen *Cladonia rangiformis* has been shown to be (+)-3*S*-methoxycarbonylheptadecane-1,2*S*-dicarboxylic acid by an NMR selective decoupling experiment with (−)-rangiformic anhydride.

One hundred years ago Paterno [1] isolated an aliphatic compound which he named (+)-rangiformic acid from the lichen *Cladonia rangiformis*. The structure and stereochemistry of its demethylation product, (+)-nor-rangiformic acid, was later elucidated as heptadecane-1,2*S*,3*S*-tricarboxylic acid by Asahina and Sasaki [2] and Åkermark [3], respectively.

Now we have localized the methyl ester group in (+)-rangiformic acid (**1**) at position 3 by ^{13}C NMR spectroscopy. (+)-Rangiformic acid gave (−)-rangiformic anhydride (**2**) on heating with acetyl chloride. The IR spectrum of **2** showed, besides the carbonyl band of the methyl ester group (1704 cm^{-1}), two strong bands at 1770 and 1844 cm^{-1} (Δ CO 74 cm^{-1}), indicative of a five-membered anhydride [4]. To differentiate between the two possible structures **2** and **3**, we performed a ^{13}C NMR double-resonance experiment with **2**. On selective decoupling at the resonance of the $-CO_2Me$ protons with varying decoupling power, the originally complex signal of the $-CO_2Me$ carbonyl group at δ 170.66 was simplified to a doublet, thus proving structure **2**. Hence, (+)-rangiformic acid is (+)-3*S*-methoxycarbonylheptadecane-1,2*S*-dicarboxylic acid (**1**).

EXPERIMENTAL

(+)-*Rangiformic acid* (**1**). **1** was obtained by extraction from *C. rangiformis Hoffm.*, collected near Varna/Bulgaria in September 1981. Needles, mp 102–104° ($MeOH$–H_2O) and $[\alpha]_D^{24} + 14.2°$ (EtOH). IR ν_{max}^{KBr} cm^{-1}: 714, 726, 842, 900, 966, 1006, 1120, 1170, 1210, 1270, 1372, 1410, 1438, 1468, 1688, 1722, 2940, 3200. MS *m/z* (rel. int.): 368 $[M - H_2O]^+$ (24), 350 (14), 336 (27), 322 (70), 308 (35), 290 (51), 280 (32), 264 (35), 228 (35), 214 (66), 210 (45), 200 (68), 196 (73), 182 (100), 168 (86), 154 (86), 140 (70), 132 (41), 126 (70), 112 (61), 98 (84), 84 (66).

(−)-*Rangiformic anhydride* (**2**). Heating **1** (0.2 g) with AcCl (20 ml) under reflux (2 hr), removal of excess AcCl (*in vacuo*) and crystallization of the residue from *n*-pentane gave **2** as plates, mp 50–51° and $[\alpha]_D^{24} - 48.1°$ ($CHCl_3$; *c* 1.175); $C_{21}H_{36}O_5$ (368.50). IR ν_{max}^{KBr} cm^{-1}: 712, 748, 884, 918, 968, 978, 1030, 1044, 1094, 1110, 1150, 1220, 1248, 1320, 1364, 1438, 1464, 1704 ($-CO_2Me$), 1770, 1844 (anhydride-CO), 2860, 2940. ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (3H, *t*–Me), 1.22 (24H, *br s*, $-(CH_2)_{12}-$), 2.30–2.90 (4H, *m*, 2 × $-CH_2-$), 2.73, 2.74, 2.77, 2.78 (1H, 2 × *d*, C-1-H), 2.90–2.99 (2H, *m*, C-2-H, C-3-H), 2.99, 3.00, 3.03, 3.05 (1H, 2 × *d*, C-1-H), 3.70 (3H, *s*, $-CO_2Me$). ^{13}C NMR (100.62 MHz, $CDCl_3$): C-1: δ 33.59; C-2: 45.44; C-3: 42.41; C-

---

*Part 139 in the series "Lichen Substances". For Part 138 see Huneck, S., Poelt, J., Ahti, T., Vitikainen, O. and Cogt, U., *Biol. Ressourcen Mongolischen Volksrepublik* (in press).