Structure Elucidation and Synthesis of Alkaloids Isolated from Fruits of *Evodia rutaecarpa*

Toshiya KAMIKADO, Shigeo MURAKOSHI^{*1,*3} and Saburo TAMURA^{*2}

Research Laboratories, Agricultural Chemicals Division, Takeda Chemical Industries, Ltd., Yodogawa-ku, Osaka ^{*1}Kanagawa Prefectural Institute for Sericultural Science, Ebina-shi, Kanagawa ^{*2}The Institute of Physical and Chemical Research, Wako-shi, Saitama

Received January 27, 1978

Five alkaloids were isolated from ripe fruits of *Evodia rutaecarpa* HOOK. f. et THOMS. Four of these were found to be indoloquinazoline type alkaloids: rutecarpine (I), evodiamine (II) and two new alkaloids, dihydrorutecarpine (IV) and its 14-formyl derivative (III). The remaining one was a new quinolone alkaloid, 1-methyl-2-nonyl-4(1H)-quinolone (V). The synthesis of II, III and IV was also achieved.

In the previous work to find insect growth inhibitors in *Evodia rutaecarpa* HOOK. f. et THOMS., we isolated three quinolone alkaloids from fresh leaves and young fruits of the plant.¹⁾ In continuation of the investigation we isolated from ripe fruits four indoloquinazoline alkaloids including two new compounds together with a new quinolone alkaloid. Further, the synthesis of the indoloquinazoline alkaloids was achieved. In this paper we wish to report the isolation, structure elucidation and synthesis of the alkaloids.

Ripe fruits of *E. rutaecarpa* were extracted with methanol. An ethyl acetate-soluble neutral fraction separated from the extract was subjected to silicic acid-celite column chromatography. The column was eluted successively with benzene, benzene-ethyl acetate and ethyl acetate. From the eluate with 5% ethyl acetate in benzene were isolated compounds I and II. The both compounds were identified as rutecarpine and evodiamine, which have been separated earlier from the same plant.^{2,3)} The eluate with 10% ethyl acetate in benzene was separated into ethyl acetate soluble and insoluble fractions. The latter fraction gave crystals, which were recrystallized from dimethylformamide-methanol to afford compound III, mp 280~281°C. The ethyl

acetate soluble fraction was applied to preparative thin-layer chromatography (TLC) using benzene-ethyl acetate (10:3) to afford compound IV, mp 214~216°C. The eluate with 40% ethyl acetate in benzene gave compound V, mp 81~82°C, together with dihydroevocarpine¹⁾ and 1-methyl-2-undecyl-4(1H)-quinolone.¹⁾

I

FIG. 1.

Compd.	R
II	CH ₃
III	CHO
IV	H

FIG. 2.

From the mass spectrum (M^+ 317) and elemental analysis the molecular formula of compound III was assigned as $C_{19}H_{15}N_3O_2$. The NMR spectrum showed no methyl protons but revealed methylene protons at δ 2.6~4.8 (4H, m), aromatic protons at δ 6.8~8.0 (8~

^{*3} Present address: Kanagawa Horticultural Experiment Station, Ninomiya, Kanagawa.

9H, m), indole NH at δ 11.05 (1H, s), and one proton at δ 9.10 (s). In the IR spectrum, two carbonyl bands were observed at 1695 and 1644 cm^{-1} . Upon heating with 5% KOH in ethanol, compound III afforded rutecarpine accompanying the loss of CH_2O . The presence of formyl group was suggested by a carbonyl band at 1695 cm^{-1} in the IR spectrum as well as by a signal at δ 9.10 in the NMR spectrum. The pattern of the UV spectrum of III with characteristic absorptions at 291 (max, $\log \epsilon$: 3.91) and 283 (shoulder, 3.93) nm was similar to that of evodiamine (II), rather than to that of rutecarpine (I). Therefore, the bond between 13b (C) and 14 (N) in III should be single. The formyl group was considered to be located on 14 (N) on the basis of chemical and spectral data and biogenetic view point.⁴⁾ This assumption was supported by the mass spectrum: m/e 288 ($\text{M}^+ - \text{CHO}$), 170 and 147 (arising from retro Diels-Alder type fragmentation of the molecular ion, see Fig. 3). Thus, the structure of compound III was elucidated to be 14-formyl-dihydrrutecarpine.

Compound IV, $\text{C}_{18}\text{H}_{15}\text{N}_3\text{O}$ (mass spectrum, M^+ 289 and elemental analysis), showed the UV spectrum similar to that of III. In the NMR spectrum, a signal at δ 6.06 (1H, s) was ascribed to a proton of 13b position. The IR spectrum revealed two NH bands at 3330 and 3280 cm^{-1} and a carbonyl band at 1633~1609 cm^{-1} . In addition, the presence of retro Diels-Alder type fragment ions, m/e 170 and 119, in the mass spectrum suggested that compound IV has dihydrrutecarpine structure.

Oxidation of IV with manganese dioxide afforded rutecarpine. Accordingly, the structure of compound IV was determined to be 13b, 14-dihydrrutecarpine (dihydrrutecarpine).

The molecular formula of compound V was determined as $\text{C}_{19}\text{H}_{27}\text{NO}$ by the mass spectrum (M^+ 285) and elemental analysis. The compound showed IR, NMR and mass spectra quite similar to those of 2-alkyl-1-methyl-4(1H)-quinolones reported in the previous paper.¹⁾ The mass spectrum revealed the base peak at m/e 173 ($\text{M}^+ - \text{C}_8\text{H}_{16}$) arising from McLafferty rearrangement of the molecular ion, together with fragment peaks with successive intervals of 14 mass units between m/e 270 ($\text{M}^+ - \text{CH}_3$) and 186 ($\text{M}^+ - \text{C}_7\text{H}_{15}$). Accordingly, the side chain in V was considered to be *n*-nonyl, and the structure of compound V must be 1-methyl-2-nonyl-4(1H)-quinolone.

Clauder *et al.*⁵⁾ already reported the synthesis of dihydrrutecarpine (IV) from N-formyltriptamine acetal and anthranilic acid, but the melting point (250~255°C, dec.) and the IR band (NH, 3250; CO, 1660 cm^{-1}) of the product in the literature were inconsistent with those of the natural dihydrrutecarpine obtained here.

For the identification, we attempted the synthesis of IV according to Clauder's method, but neither IV nor the product reported in the literature were obtainable. Kametani *et al.*³⁾ prepared rutecarpine (I) in one step from 3,4-dihydro- β -carboline and 3,2,1-benzoxathiazin-4(1H)-one 2-oxide (VI). Then, we treated isatoic anhydride, in place of VI, with 3,4-dihydro- β -carboline in dry benzene-pyridine at

FIG. 3. Retro Diels-Alder Type Fragment Ions Observed in the Mass Spectra of Three Indoloquinazoline Alkaloids.

^a Figures in parentheses represent relative intensities of the ions.

FIG. 4.

60°C and successfully obtained IV in 71.5% yield. Oxidation of the product with manganese dioxide gave rutecarpine. The NMR and mass spectra of synthetic IV were identical with those of the natural product. However, the melting point (227~230°C) of the synthetic sample was higher than that (214~216°C) of the natural one. Further, the IR spectra (KBr) of the both samples were inconsistent in some respects (Fig. 5). These might be due to the formation of racemic compound in the crystals of synthetic IV.

The above reaction was also applied to the synthesis of evodiamine (II). Treatment of 3,4-dihydro- β -carboline with N-methylisatoic anhydride at 55°C gave II in 66% yield. The physicochemical data of the product were identical with those of the natural sample.

Formylation of IV with acetic formic an-

hydride in pyridine were unsuccessful. When the reaction was carried out in formic acid, however, 14-formyldihydorrutecarpine (III), mp 281~282°C, was obtained in 79% yield. The NMR and mass spectra of the product were identical with those of the natural product, while some inconsistency was observed in the IR spectra (KBr, see EXPERIMENTAL).

It has been reported that indoloquinazoline alkaloids are biosynthesized from tryptophan and anthranilic acid.⁴⁾ 14-Formyldihydorrutecarpine is supposed to be an oxidation product of evodiamine in this biogenetic system, and dihydorrutecarpine may be an intermediate product in the biosynthesis of rutecarpine.

The absolute configurations of the indoloquinazoline alkaloids in this paper remain to be elucidated.

Every alkaloid obtained here showed growth-inhibitory effect on silkworm larvae by oral administration. Details of the biological activities will be reported elsewhere.

EXPERIMENTAL

All melting points are uncorrected. UV spectra were determined on a Hitachi EPS-3T Recording Spectrophotometer. IR spectra were recorded on a Hitachi EPI-G3 Grating Infrared Spectrophotometer. NMR spectra were measured with Varian XL-100 and HA-100 NMR Spectrometer. The data were obtained at 100 MHz and indicated by δ values from tetramethylsilane as a standard. Mass spectra were recorded with a JMS-01SG Mass Spectrometer. Thin-layer chromatography (TLC) was conducted by use of Silica gel

FIG. 5. IR Spectra of Dihydorrutecarpine (KBr).
 —, natural product; ----, synthetic sample.

GF₂₅₄ Type 60 (Merck), and preparative TLC was carried out on plates of the same materials (0.5 mm thickness). For reversed-phase TLC, Silica gel GF₂₅₄ plates were immersed for 2 min in a solution of 5% silicone (Shimadzu, Silicone D.C. 200) in ether and the siliconized plates were used immediately after evaporation of the solvent. Spots on chromatograms were detected with a UV lamp and Dragendorff reagent. Column chromatography was performed by use of Silica gel 60 (Merck) and Celite 545 (Johns-Manville).

Separation of neutral fraction

Ripe fruits (300 g) of *E. rutaecarpa* were collected at Kyoto Herbal Garden of Takeda Chemical Industries, Ltd., early in December of 1975. The fruits were extracted with two 1000 ml-portions of methanol in a blender, and the combined extracts were evaporated under reduced pressure. The residue was extracted with three 100 ml-portions of ethyl acetate at pH 3. The combined extracts were shaken with two 80 ml-portions of saturated aq. NaHCO₃, dried over anhyd. Na₂SO₄ and filtered. The filtrate was evaporated under reduced pressure to give a neutral fraction as a dark-green oil (4.9 g).

Isolation of alkaloids

The neutral fraction (4.9 g) was dissolved in 50 ml of acetone and was shaken with 5 g of celite. After evaporation of the solvent under reduced pressure, the resulting powder was applied onto a column (43 × 200 mm) packed with silica gel-celite (90 g + 30 g). The column was successively eluted with 1000 ml each of benzene, benzene-ethyl acetate (9.5:0.5; 9:1; 8:2; 6:4) and ethyl acetate. Each eluate was evaporated under reduced pressure to give oils. Yields were as follows: 842, 827, 908, 891, 628, 253 mg in the order of elution.

Isolation of rutecarpine (I) and evodiamine (II). The oil (40 mg) from the eluate with 5% ethyl acetate in benzene was subjected to preparative TLC by the use of silicic acid and benzene-ethyl acetate (4:1). The crude crystals (9 mg) obtained from a zone at *Rf* 0.58 were recrystallized from benzene-ethyl acetate to give compound I (rutecarpine) as pale yellow needles, mp 263~264°C (Lit.²⁾ 256°C, Lit.³⁾ 259°C). MS *m/e*: 287 (M⁺). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3345, 1653, 1600, 1475, 1408, 1335, 1237, 772, 764, 738, 731. NMR $\delta_{\text{TMS}}^{\text{d}_6\text{-DMSO}}$: 3.18 (2H, t, *J*=7Hz), 4.45 (2H, t, *J*=7Hz), 7.0~7.9 (7H, m), 8.18 (1H, d, *J*=8Hz), 11.85 (1H, br s).

The crude crystals (25 mg) obtained from a zone at *Rf* 0.48 were recrystallized from benzene-ethyl acetate to give compound II (evodiamine) as colorless leaflets, mp 268~272°C (Lit.²⁾ 270~272°C). MS *m/e*: 303 (M⁺), 170, 169, 133, 134. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3225, 1640, 1628, 1605, 748, 736. NMR $\delta_{\text{TMS}}^{\text{d}_6\text{-DMSO}}$: 2.65~3.2 (3H, m), 2.90 (3H, s), 4.67 (1H, d, *J*=10 Hz, 3 Hz), 6.12

(1H, s), 6.9~7.65 (7H, m), 7.83 (1H, d, *J*=9 Hz, 2 Hz), 11.05 (1H, s).

Isolation of compounds III and IV. The oil (900 mg) from the eluate with 10% ethyl acetate in benzene was dissolved in 20 ml of ethyl acetate and insoluble solids (350 mg) were collected by filtration. The solids (300 mg) were recrystallized twice from methanol-dimethylformamide to give compound III (109 mg) as colorless needles, mp 280~281°C. MS *m/e*: 317 (M⁺), 288, 287 (base peak), 170, 169, 148, 147. Anal. Found: C, 71.95; H, 4.71; N, 13.12. Calcd. for C₁₉H₁₅N₃O₂: C, 71.98; H, 4.77; N, 13.26%. $[\alpha]_{\text{D}}^{25} + 260^\circ$ (*c*=0.535, DMF). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 291 (3.91), 283 (sh, 3.98). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3265, 1695, 1644, 1606, 1491, 1385, 1288, 1240, 1230, 765 (sh), 758, 741, 707, 692, 640. NMR $\delta_{\text{TMS}}^{\text{d}_6\text{-DMSO}}$: 2.6~3.7 (3H, m), 4.72 (1H, d, *J*=12.5 Hz, 5 Hz), 6.8~7.7 (8H, m), 7.92 (1H, d, *J*=8 Hz), 9.1 (1H, s), 11.05 (1H, br s).

The ethyl acetate soluble fraction was evaporated under reduced pressure to give an oil (547 mg), which was subjected to preparative TLC by the use of silicic acid and benzene-ethyl acetate (10:3). The crystals (79 mg) obtained from a zone at *Rf* 0.46 were rechromatographed with the same system and then recrystallized from ethyl acetate to afford compound IV (26 mg) as pale yellow prisms, mp 214~216°C. MS *m/e*: 289 (M⁺), 288 (base peak), 287, 170, 169, 120, 119. Anal. Found: C, 74.39; H, 5.03; N, 13.92. Calcd. for C₁₈H₁₅N₃O: C, 74.71; H, 5.22; N, 14.35%. $[\alpha]_{\text{D}}^{25} - 564^\circ$ (*c*=0.355, DMF). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 290 (3.91), 281 (4.03). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3330, 3280, 1633, 1618 (sh), 1609, 1425, 760 (sh), 750, 735 (see Fig. 5). NMR $\delta_{\text{TMS}}^{\text{d}_6\text{-DMSO}}$: 2.65~3.4 (4H, m), 4.83 (1H, d, *J*=13 Hz, 3 Hz), 6.06 (1H, s), 6.7~7.6 (7H, m), 7.80 (1H, d, *J*=8 Hz, 2 Hz), 10.9 (1H, br s).

Isolation of compound V. The yellowish green oil (160 mg) from the eluate with 40% ethyl acetate in benzene was dissolved in 20 ml of dichloromethane. The solution was added with *m*-chloroperbenzoic acid (130 mg) and stirred for 4 hr at room temperature. The reaction mixture was washed with aq. NaHCO₃, dried over anhyd. Na₂SO₄ and evaporated under reduced pressure to give an oil (150 mg). The oil was applied to preparative TLC using silicic acid and ethyl acetate. A zone at *Rf* 0.38 was extracted with ethyl acetate and evaporated to give a quinolone alkaloid mixture as a colorless oil. The mixture (56 mg) was applied to preparative reversed-phase TLC using siliconized silicic acid plates and acetonitrile-acetic acid-water (7:3:5). The silica gel powder collected from a zone at *Rf* 0.68 was washed with ether and extracted with ethyl acetate. The extract was washed with 10% aq. NaHCO₃, dried over anhyd. Na₂SO₄ and

evaporated to afford crude crystals of compound V (9.7 mg), which were recrystallized from ethyl acetate-ether to give colorless leaflets melting at 81~82°C.

Dihydroevocarpine (4 mg) and 1-methyl-2-undecyl-4 (1H)-quinolone (19 mg) were also obtained from zones at *Rf* 0.40 and *Rf* 0.54, respectively.

Alkaline cleavage of compound III

To a solution of 0.25 g of potassium hydroxide in 5 ml of 95% ethanol was added 13 mg of compound III. The mixture was heated under reflux for 6 hr. After cooling, the separated crystals were collected and dried to give 4.2 mg of needles, mp 262~264°C. Concentration of the filtrate gave further 5 mg of crystals (mp 262~264°C). The both specimens were found to be identical with compound I (rutecarpine) through mixed melting point determination and comparisons of IR spectra.

Oxidation of compound IV

To a solution of 6.5 mg of compound IV in 2 ml of acetone was added 25 mg of manganese dioxide. The mixture was stirred for 6 hr at room temperature and allowed to stand overnight. After filtration of the mixture, the filtrate was concentrated to dryness. The residue was recrystallized from ethyl acetate-acetone to give 4 mg of colorless needles, mp 264°C. The product was found to be identical with compound I by mixing melting point and spectral measurement.

Synthesis of dihydrorutecarpine (IV)

To a mixture of 3,4-dihydro- β -carboline (173 mg)⁶⁾ and isatoic anhydride (163 mg) in dry benzene was added dry pyridine (0.5 ml), and the mixture was warmed to 60°C. The crystals of isatoic anhydride dissolved with evolution of carbon dioxide and then new crystals were deposited. After stirring for 30 min at 60°C, the reaction mixture was cooled to 10°C and filtered to give the crystals (186 mg) of IV, which were recrystallized from ethyl acetate-acetone to afford colorless needles melting at 227~230°C. *Anal.* Found: C, 74.49; H, 5.04; N, 14.33. *Calcd.* for $C_{18}H_{15}N_3O$: C, 74.71; H, 5.22; N, 14.35%. IR (see Fig. 5). The NMR and mass spectra of the product were identical with those of the natural product.

Synthesis of evodiamine (II)

A mixture of 3,4-dihydro- β -carboline (61 mg), N-methylisatoic anhydride (62 mg) and pyridine (1 ml) in dry benzene (4 ml) was stirred for 2 hr at 55°C and

filtered. On cooling, crystals appeared in the filtrate. The crystals were collected and washed with benzene to give II (57.1 mg). Concentration of the mother liquor followed by recrystallization from dimethylformamide-acetone afforded further 12.5 mg of II. Mp 269~271°C. *Anal.* Found: C, 74.95; H, 5.61; N, 13.62. *Calcd.* for $C_{19}H_{17}N_3O$: C, 75.22; H, 5.65; N, 13.85%. The IR, NMR and mass spectra of the product were identical with those of the natural product. The mixture melting point with the natural product showed no depression.

Synthesis of 14-formyldihydrorutecarpine (III)

A mixture of acetic anhydride (0.2 ml) and formic acid (0.3 ml) was warmed for 10 min at 55°C and cooled to room temperature. Dihydrorutecarpine (15 mg) was added to the mixture and stirred for 2 hr. The solution was concentrated to dryness under reduced pressure and the residue was washed with a small amount of cold methanol to give 13 mg of III. Mp 281~282°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3320, 1688, 1640, 1605, 1491, 1375, 1283, 1225 (sh), 769, 758, 742, 700, 660, 630. The NMR and mass spectra of the product were identical with those of the natural product. The mixture melting point with the natural product showed no depression.

Acknowledgement. The authors wish to express our thanks to Mr. T. Takahashi of the Pharmacognotic Research Laboratories of Takeda Chemical Industries, Ltd. for providing the plant materials. We also thank Dr. K. Ono of Agricultural Chemicals Division of the Company and Mr. K. Morii of Kanagawa Prefectural Institute for Sericultural Science for their cooperation.

REFERENCES

- 1) T. Kamikado, C.-F. Chang, S. Murakoshi, A. Sakurai and S. Tamura, *Agric. Biol. Chem.*, **40**, 605 (1976).
- 2) T. Nakasato, S. Asada and K. Marui, *Yakugaku Zasshi*, **82**, 619 (1961).
- 3) T. Kametani, T. Higa and K. Fukumoto, *Heterocycles*, **4**, 23 (1976).
- 4) M. Yamazaki, A. Ikuta, T. Mori and T. Kawana, *Tetrahedron Lett.*, **1967**, 3317.
- 5) O. Clauder and K. Horváth-Dóra, *Acta Chim. (Budapest)*, **72**, 221 (1972).
- 6) Y. Kanaoka, E. Sato and Y. Ban, *Chim. Pharm. Bull.*, **15**, 101 (1967).