ANTITUMOR PLANTS. X.[1,2] CONSTITUENTS OF *NECTANDRA RIGIDA*

PHILIP W. LE QUESNE, JESUS E. LARRAHONDO and ROBERT F. RAFFAUF

Department of Chemistry and Department of Medicinal Chemistry and Pharmacology, Northeastern University, Boston, Massachusetts 02115

ABSTRACT.—The major cytotoxic activity of crude extracts of *Nectandra rigida* Nees is due to dehydrodiisoeugenol (**1**), the sample isolated being slightly enriched in the dextrorotatory enantiomer. Galgravin (**4**) and two new tetrahydrofuranoid lignans, nectandrin A (**5**) and nectandrin B (**8**), were also isolated and characterized along with small quantities of vanillin, 2,6-dimethoxybenzoquinone, and the known lauraceous alkaloid laurelliptine (**9**). The neolignans are of potential chemotaxonomic significance in the study of the Lauraceae.

The Lauraceae, a predominantly arboreal family of 50–60 genera and 1100 species, has principal centers of distribution in Southeast Asia and Brazil. Many species have been used as timber, perfume, and spices; secondary metabolites have been of considerable phytochemical interest (1, 2). In recent years, some species have been shown to demonstrate potential antitumor activity, e.g., *Litsea turfosa* (3) and *Aniba megaphylla* (4). We now report our investigation of the chemical constituents of *Nectandra rigida*, another species of interest by virtue of the cytotoxicity of its extracts in the KB assay (5).

Dried, ground leaves and stems were extracted and partitioned in the usual manner (6). The aqueous fraction (A), which was of considerable bulk, was inactive *vs.* KB. It gave a positive Dragendorff test for alkaloids and, on this basis, was partitioned into alkaloidal and nonalkaloidal portions in the usual way. The alkaloidal material was isolated and identified as laurelliptine; the isolation and identification of this same material from the methanol-water (9:1) fraction is described fully below. The nonalkaloidal material gave positive qualitative tests for saponins, sterols, tannins, and reducing sugars, but was not examined in further detail.

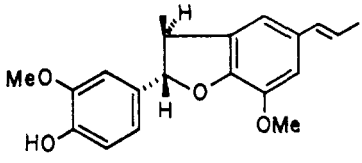
The cytotoxicity observed in crude extracts was found in the methanol-water (9:1) fraction, which showed ED_{50} *vs.* KB at 29 μg/mL, and which also contained alkaloids (Dragendorff test); partition into alkaloidal and nonalkaloidal subfractions concentrated the activity in the latter.

Chromatography of the nonalkaloidal portion on silica gel (gradient elution) followed by further chromatographic sub-separation, yielded several compounds. The first of these, the major constituent, mp 118–120°, had a molecular formula $C_{20}H_{22}O_4$ and displayed ED_{50} *vs.* KB at 7.0 μg/mL. Its spectral characteristics suggested identity with the neolignan licarin A (**1**) (relative configuration only is implied (7)). Licarin A, $[\alpha]_D$ −59° (8) is an optically active form of dehydrodiisoeugenol, which was proposed long ago by Erdtman (9) to be an intermediate in lignin biosynthesis. The chemical identity of our compound, $[\alpha]_D$ +11.4°, with (±)-dehydrodiisoeugenol was established by synthesis of the latter (10) and direct comparison with an authentic sample. Our material and licarin A are apparently enantiomerically enriched samples of dehydrodiisoeugenol, an interest-

[1]For Part IX, see P. W. Le Quesne, M. D. Menachery and R. F. Raffauf, *J. Nat. Prod.*, **42**, 320 (1979).

[2]The word "Antitumor" as used in this title signifies no more than the fact that his plant was regarded by the National Cancer Institute as being of sufficient potential interest in this respect to warrant investigation.

ing circumstance which suggests differences in the enzymatic enantioselectivity of closely related botanical taxa in their biosynthesis of neolignans. We attempted to determine the optical purity and also establish the specific rotation of the optically pure enantiomers by using the chiral shift reagent Eu(hfc)$_3$ (11). This attempt was unsuccessful; addition of the reagent in gradually increasing molar



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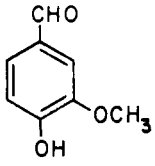

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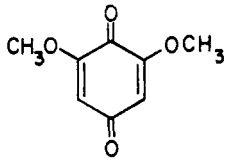

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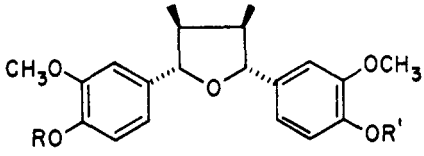

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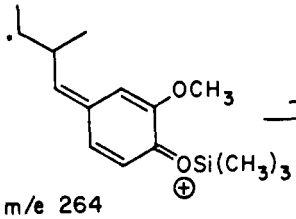

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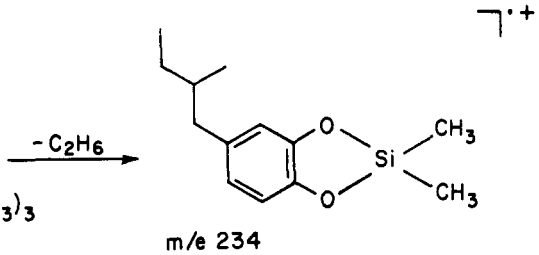

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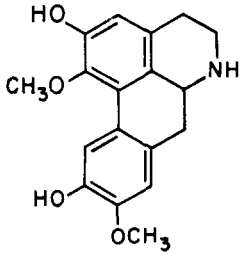

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ratios led only to broadening of the nmr signals of potential interest. In view of the cytotoxicity of our dextrorotatory sample of dehydrodiisoeugenol, we considered it of potential value to assess the relative biological activities of both enantiomers of this compound; the opportunity to do this is very seldom available in the area of naturally occurring antitumor agents. A preliminary attempted resolution of the synthetic racemate *via* the menthoxyacetates (12, 13) was unsuccessful, but our interest in this aspect of the work continues.

The compounds following dehydrodiisoeugenol in order of increasing polarity were vanillin (**2**), and a yellow compound, $C_8H_8O_4$, mp 204° (dec.), identified as 2,6-dimethoxybenzoquinone (3), which had been isolated previously by Karrer from *Adonis vernalis* (14).

Three additional compounds were obtained in small quantities by continued gradient elution. They were the two new oily compounds nectandrin A, $C_{21}H_{26}O_5$, $[\alpha]D+10°$, nectandrin B, $C_{20}H_{24}O_5$, $[\alpha]D$ 0°, and a crystalline compound, $C_{22}H_{28}O_5$, mp 116–118°, $[\alpha]D$ 0°. Their nmr and mass spectra were characteristic of tetrahydrofuranoid lignans, and the crystalline compound was identified as galgravin (**4**) by direct comparison with authentic material. Galgravin had been isolated previously from the closely related lauraceous species *Ocotea veraguensis* (15) and from *Magnolia acuminata* (Magnoliaceae) (16). Both nectandrin A and B on methylation yielded galgravin, showing that they are, respectively, mono- and di-phenolic compounds. Structure **5** is assigned to nectandrin A from these data and on the basis of its likely biogenesis from isoeugenol. In nectandrin B each phenolic group was shown to be adjacent to a methoxy-group by the characteristic mass spectral fragmentation pattern of the bis(trimethylsilyl) ether. Mass spectral analysis showed the structural symmetry of both the parent compound and this derivative (17); in addition, the loss of ethane from daughter ion **6**, proceeding as shown to give **7**, confirmed the adjacency of the methoxy- and trimethylsilyloxy-groups. This fragmentation was confirmed by observations with the bis(perdeuterotrimethylsilyl) derivative. From these data, and assuming biogenesis from iso-eugenol, we assign structure **8** to nectandrin B.

The alkaloidal fraction mentioned above was refined by chromatography over alumina and then by preparative tlc. The predominant component, obtained in small amount, was identified by ultraviolet spectroscopy and mass spectra (the latter employing derivatization as the *bis*-(*O*-trimethylsilyl)-*N*-heptafluorobutyryl derivative), and finally by direct comparison with the noraporphine alkaloid laurelliptine (**9**). This alkaloid had been obtained previously from the Australian lauraceous species *Beilschmiedia elliptica* (18).

β-Sitosterol was characterized as a constituent of the light petroleum soluble fraction of the original extract of *N. rigida*.

The major cytotoxic activity of the plant appears to arise from the presence of dehydrodiisoeugenol whose activity in this respect has not been reported previously. This compound and the other constituents isolated are also of potential importance in the chemotaxonomic study of this section of the Lauraceae.

EXPERIMENTAL[3]

EXTRACTIONS AND PARTITIONS.—The dried, ground leaves and stems (6.4 Kg) of *N. rigida* were extracted to exhaustion at room temperature with 95% ethanol. The alcohol was removed under reduced pressure below 45°, and the syrup remaining after evaporation was partitioned between chloroform (14 x 1 liter) and methanol-water (1:9, 14 x 1 liter). During the partition, apparently polymeric interface material (88 g) formed and was removed by filtra-

[3]General experimental directions are given in Part IV of this series; see Ref. (6).

tion. It was not further investigated. The aqueous fraction when concentrated to small volume (rotary evaporator) and, finally, freeze-dried gave a brown solid (A) (527 g). The chloroform fraction was concentrated to a syrup and partitioned between light petroleum (5 x 1 liter) and methanol-water (9:1, 5 x 1 liter). Concentration of these extracts gave, after freeze-drying, a methanol fraction (B) (44.5 g) and, after removal of the solvent under reduced pressure, a light petroleum fraction (C) (106.9 g).

CONSTITUENTS OF THE AQUEOUS FRACTION (A).—A sample of the aqueous fraction (freeze-dried powder, 0.2 g), when dissolved in ethanol (3 ml), gave positive tests with Dragendorff's and Mayer's reagents showing the presence of alkaloids. The solution remaining after basification with ammonia and extraction with chloroform reacted negatively to these tests indicating the absence of quaternary alkaloid derivatives. An aqueous solution of the fraction gave positive tests for saponins (frothing), tannins (gelatin and ferric chloride), sterols (Liebermann-Burchard), and reducing sugars (Fehling).

The alkaloidal constituents of the fraction were obtained as follows. Fraction (A) (519 g) was treated with 2% aqueous HCl (2 liters). After filtration, the solution was basified with ammonia and extracted with chloroform (5 x 1 liter). The chloroform extract was washed with distilled water and dried (sodium sulfate), then filtered and concentrated under reduced pressure to give a crude alkaloidal residue (0.31 g, positive Dragendorff test). The alkaloids so obtained were further purified by preparative tlc (aluminum oxide, benzene-acetone-ammonia, 10:10:0.5). The predominant alkaloid obtained was laurelliptine (**9**) (8 mg), also obtained from the methanolic fraction (B) (see below). The uv spectrum, mass spectrum [*m/e*=313 (M^+) (80), 312 (100), 298 (17), 284 (15), 269 (7), 254 (7), 253 (11)], and co-tlc (silica gel) (acetone-methanol, 3:2; chloroform-methanol, 17:3) with an authentic sample confirmed the identity.

CONSTITUENTS OF THE METHANOLIC FRACTION (B).—This fraction showed activity *vs.* KB (ED_{50}=29 μg/ml) and gave a positive test for alkaloids (Dragendorff). The fraction (42.3 g) was suspended in tartaric acid (5%, 400 ml) and extracted with chloroform (3 x 500 ml). Plant dust brought through from the initial extraction was removed by filtration under vacuum through Celite; the chloroform was washed with water (3 x 400 ml), dried (sodium sulfate) and concentrated under reduced pressure to a green syrup (27.5 g). The aqueous layer was made basic (pH=9) with ammonia and extracted with chloroform (4 x 500 ml). The chloroform layer was washed with water (3 x 500 ml), dried (sodium sulfate), and freed of solvent under reduced pressure to give an oily, Dragendorff-positive residue (500 mg).

a. *Nonalkaloidal Portion.*

This fraction (27.5 g) was chromatographed on silica gel (Woelm, activity III, 800 g), eluted with hexane, benzene, chloroform, and methanol. Six fractions were collected; these and their associated activities *vs.* KB are listed in table 1.

TABLE 1. Weights and KB activities of nonbasic methanol-soluble extractives.

Fraction	Solvent	Weight (g)	ED_{50} μg/ml (KB)
1	Hexane	0.10	>100
2	Benzene	0.20	>100
3	Chloroform	0.81	31
4	"	3.45	18
5	"	0.69	29.5
6	Methanol	17.9	>100

Chromatography of fraction 3 on four silica gel preparative plates (E. Merck, 0.5 mm; benzene-ethyl acetate, 98:2) gave, after excision and extraction, one major component (100 mg) which crystallized from ether as needles, mp 118–120°; $[\alpha]^{26}D$ +11.4 (c=1.1, MeOH); ir (KBr) 3360, 1610, 1510, 1490, 1450, 1335, 1275, 1220, 1150, 1130, 1075, 960, 900, 860, 810 cm^{-1}; uv λmax (MeOH) 220 (ε 35000) and 272 nm (ε 27000); ^1H nmr ($CDCl_3$) δ 7.0–6.7 (5H, m), 6.4 (1H, d, *J*=15 Hz), 6.1 (1H, dq, *J*=15, 5 Hz), 5.6 (1H, s, OH exchangeable with D_2O), 5.1 (1H, d, *J*=10 Hz), 3.9 (6H, s, 2 OCH_3), 3.4 (1H, dq, *J*=9, 6 Hz), 1.9 (3H, d, *J*=5 Hz), 1.4 (3H, d, *J*=7 Hz); ^{13}C nmr ($CDCl_3$) δ 17.7, 18.0, 46.6, 56.0, 94.4, 110.0, 112.0, 114.0, 116.0, 122.0, 125.6, 132.0, 133.2, 134.0, 143.4, 146.4, 146.7, 147.6 ppm. Mass spectrum at high resolution indicated *m/e* 326.14864 (M^+ calcd. for $C_{20}H_{22}O_4$, 326.15181); mass spectrum at low resolution indicated *m/e* 326 (M^+, 100), 311 (14), 283 (6), 202 (11), 189 (6), 151 (7), 149 (6), 137 (8), 131 (3). Except for its optical activity, this compound was identical in all respects to a sample of (±)-dehydrodiisoeugenol (**1**) prepared synthetically according to the literature (10).

Fractions 4 and 5, which appeared very similar on analytical tlc (silica gel, benzene-ethyl acetate, 98:2), were combined and chromatographed on silica gel (Woelm, activity III, 140 g) with gradient elution *via* benzene, benzene chloroform (98:2, 95:5, 9:1, 3:1, 1:1), chloroform, chloroform-methanol (98:2, 95:5, 9:1, 3:1, 1:1, 3:7), and methanol. Thirty-four fractions of 50 ml each were collected. Many of the fractions comprised oily mixtures from which no homogeneous compounds could be characterized. However, from fractions 10–15 (benzene-chloroform 1:1) solid material was obtained which, after repeated crystallization from methanol, gave vanillin (**2**) (30 mg), mp 78–81°; lit. (19) mp 81°. Identity was confirmed by direct comparison with authentic material. From fraction 20 (benzene-chloroform, 3:7) a brown oil (67 mg) was obtained which, after trituration and repeated crystallization from ethyl acetate-toluene, yielded yellow needles (2 mg), mp 240° (dec.); ir ($CHCl_3$) 1690, 1640, 1620, 1590, 1110, 880 cm^{-1}; uv λmax (MeOH) 286 nm (ε 5300); ^1H nmr ($CDCl_3$) δ 3.9 (6H, s), 6.3 (2H, s); mass spectrum *m/e* 168 (M^-, 100), 140 (14), 138 (38), 125 (20), 80 (49). This material gave a blood-red color in concentrated sulfuric acid. The absorption spectrum reported for 2,6-dimethoxybenzoquinone (**3**) (20) was identical to that of our natural product.

Fractions 28 and 29, collected after elution with chloroform, were concentrated under reduced pressure to give green oily residues (0.77 and 0.39 g, respectively). Further purification of both fractions by preparative tlc (silica gel Merck, 0.5 mm, benzene-ethyl acetate, 98:2) yielded, after excision and extraction (chloroform-methanol, 98:2), a pale yellow oil as the major component of the mixtures (32 mg). This oil was obtained in better yield in a second extraction of bark, leaves and stems of the plant (see below) in order to complete the characterization. Several attempts to crystallize it were unsuccessful. The pure compound, nectandrin A (**5**), had $[\alpha]^{24}D+10°$ (c=1.4, MeOH); ir (film): 3400, 2960, 1610, 1600, 1520, 1460, 1430, 1380, 1270, 1240, 1030, 860, 800, 770 and 750 cm^{-1}; uv λmax (MeOH): 278, 231nm (ε 5600, 17000); a shift to 294 nm (ε 18000) was observed on addition of 1–2 drops of 2% NaOH solution. The ^1H nmr ($CDCl_3$) showed signals at δ 6.8–7.0 (6H, s), 4.5 (2H, d, J=6 Hz), 3.9 (9H, s), 2.3 (2H, m), 1.1 (6H, d J=6 Hz); a ^{13}C nmr ($CDCl_3$) proton-noise-decoupled (PND) spectrum showed chemical shifts at 12.9, 44.3, 55.9, 87.2, 109.3, 109.4, 110.1, 110.7, 118.6, 119.3, 134.9 and 145.1 ppm.

High resolution mass measurements gave *m/e* at 358.17648 (M^+, calcd. for $C_{21}H_{26}O_5$, 358.17802). Other fragments observed in the mass spectrum were 206 (64), 192 (90), 177 (25), 175 (24), 151 (18) and 145 (23). All spectral data were similar to, but not identical with, those reported for galgravin (**4**) (15).

TRIMETHYLSILYLATION OF NECTANDRIN A.—*Bis*-trimethylsilyltrifluoroacetamide (BSTFA) (30 μl) was added to a solution of (**5**) (1 mg) in anhydrous ethyl acetate (30 μl), and the reaction mixture was held at 80° for 1 hr. An aliquot (~5 μl) was used for mass spectral analysis. The spectrum exhibited peaks at *m/e* 430 (M^-, 25), 264 (50), 249 (15), 235 (18), 234 (23), 206 (100), 191 (40), 177 (37), 73 (20). When nectandrin A was similarly treated with BSTFA-d_9, mass shifts to 439, 273, 243, and 240 were observed.

METHYLATION OF NECTANDRIN A.—Alternate addition of dimethyl sulfate (6 mg) and aqueous sodium hydroxide (10%, 2 ml) to a solution of **5** (12 mg) in ethanol (2 ml) was performed with continuous magnetic stirring for 14 hr. The solution was extracted with ether. The ether extract was dried over sodium sulfate, filtered, and evaporated. The product crystallized from ether as fine needles of **4**. Mass spectral analysis gave fragments at *m/e* 372 (M^-, 48), 206 (100), 194 (5), 191 (37), 178 (10), 175 (35), 168 (8), 166 (5). The same product was obtained by treatment of **5** (1 mg) with diazomethane in ether with a catalytic amount of aluminum chloride. The mass spectrum of this preparation was also in agreement with that given for galgravin (**4**) in the literature. Identity was confirmed by tlc and mixed tlc (silica gel, toluene-ethyl acetate, 9:1) with an authentic sample.

Fraction 30, from the chromatography of fractions 4 and 5 (table 1), collected after elution with chloroform-methanol (98:2), was concentrated to give an oily material (413 mg) which, after several purifications by preparative tlc (silica gel, chloroform-methanol, 98:2), gave a pale yellow oil (10 mg). An additional quantity of the compound (15 mg) was also obtained in a second extraction of the plant. Several attempts at crystallization were unsuccessful. The oily compound, nectandrin B, was homogeneous by tlc analysis in different systems (chloroform-methanol, 98:2; toluene-ethyl acetate, 9:1). It had $[\alpha]D0°$ (MeOH), and ir (film) 3400, 2950, 1620, 1520, 1460, 1260, 1240, 880, 820, and 790 cm^{-1}; uv λmax (MeOH) 278 and 230 nm (ε 4000, 10000); on addition of 2% NaOH a shift to 290 and 250 nm (ε 4400, 11000) was observed; ^1H nmr ($CDCl_3$) δ 1.0 (6H, d, J=6 Hz), 2.3 (2H, m), 3.9 (6H, m); mass spectrum, M^- 344.16321; calcd. for $C_{20}H_{24}O_5$, 344.16237; *m/e* 344 (M^+, 19), 192 (100), 177 (32), 164 (10), 161 (12), 152 (10), 145 (36), 137 (8).

TRIMETHYLSILYLATION OF NECTANDRIN B.—A sample (1 mg) of **8** was treated with BSTFA (30 μl) in anhydrous ethyl acetate (30 μl) at 80°. After 1 hr an aliquot (4 μl) was used for mass spectral analysis. The fragmentation pattern displayed the following peaks: *m/e* 488 (M^-, 25), 473 (8), 264 (100), 249 (20), 234 (18), 233 (35), 223 (8), 219 (8), 73 (10). Under conditions similar to these, nectandrin B was also treated with BSTFA-d_9, and mass shifts in the spectrum of the product were seen at *m/e* 506, 273, 258, 242 and 240.

METHYLATION OF NECTANDRIN B.—Methylation of **8** (5 mg) was performed with dimethyl sulfate (7 mg) in aqueous NaOH (10%) under conditions identical to those described for nectandrin A. The mass spectrum of the product exhibited fragments at *m/e* 372 (M^+, 48), 206 (100), 191 (37), 175 (35). The pattern is in agreement with literature data (15) for galgravin (**4**). Identity was confirmed by tlc and co-tlc comparison with an authentic sample.

SECOND EXTRACTION OF *N. rigida* AND ISOLATION OF GALGRAVIN (**4**).—A second extraction of dried, ground bark and stems (5 kg) of *N. rigida* with ethanol, followed by concentration of the extract under reduced pressure, yielded a syrup (462 g) which was partitioned according to the procedure described previously. The methanolic fraction (B, 40 g) was extracted with benzene, and the soluble portion was concentrated to a green syrup (27 g).

Chromatography of this material on silica gel (Woelm, activity III, 700 g) with toluene, toluene-ethyl acetate (98:2, 95:5, 9:1, 3:1, 1:1) and ethyl acetate, gave additional dehydrodiisoeugenol (**1**) (406 mg), crystallized from ether after elution and concentration from the toluene-ethyl acetate (98:2) fraction. The fractions eluted with toluene-ethyl acetate 9:1 were concentrated to a brown oily material (1.3 g) and refined by chromatography on silica gel (40 g) by use of the same solvent system. Careful collection of the initial fractions yielded, after concentration and crystallization from ether, colorless crystals (26 mg) of galgravin (**4**); mp 116–118°; $[\alpha]^{26}D$ 0° (c=0.7, MeOH) [lit. (15), mp 121°, $[\alpha]^{22}D$ 0° (c=1.30, $CHCl_3$)]; ir (KBr) 3000, 2960, 2940, 2880, 2840, 1610, 1590, 1510, 1460, 1260, 1240, 1160, 1140, 1030, 860 and 800 cm^{-1}; uv λmax 232 and 278 nm (ε 4500, 15000); ^1H nmr ($CDCl_3$) displayed signals at δ 1.1 (6H, d, *J*= 6 Hz), 2.3 (2H, m), 3.9 (12H, s), 4.5 (2H, d, *J*=6 Hz), 6.9–7.1 (6H, m). Mass spectrum at high resolution indicated *m/e* 372.19367 (M^+, calcd. for $C_{22}H_{28}O_5$, 372.18780; mass spectrum at low resolution indicated *m/e* 372 (M^+, 48), 206 (100), 191 (37), 178 (10), 175 (35), 165 (9). These data were in agreement with those reported for galgravin (15). Tlc and mixed tlc (silica gel, toluene-ethyl acetate, 9:1) with an authentic sample confirmed the identity.

Additional amounts of nectandrin A (70 mg) and nectandrin B (15 mg) were obtained after concentration of the last fractions collected by elution with toluene-ethyl acetate 9:1. Both compounds were purified by preparative tlc on silica gel (activity III) eluting twice with chloroform-methanol (98:2) to give **5** (R_f 0.47) and **8** (R_f 0.23) as oils. Nectandrin A, nectandrin B and galgravin developed intense red colors with concentrated sulfuric acid, permitting excellent visualization on tlc plates and detection by spot test (~0.1 mg sample, 2 drops H_2SO_4).

b. *Alkaloidal Portion.*

The oily material (500 mg) was refined by chromatography on alumina (Woelm, activity III, 25 g) by gradient elution *via* benzene, benzene-chloroform (99:1, 98:2, 95:5, 9:1, 3:1 and 1:1), chloroform, chloroform-methanol (98:2, 95:5, 9:1, 3:1 and 1:1), and methanol. Each fraction (20 ml) was examined by tlc (alumina) and concentrated under reduced pressure. Thirty-four fractions were collected. Fractions 1–21 showed three Dragendorff-negative spots on examination by tlc (alumina, chloroform-methanol (95:5)). Fractions 21–25 (chloroform-methanol, 98:2, 149 mg) and 26–30 (chloroform-methanol, 95:5, 9 mg) indicated 2 Dragendorff-positive spots. Fractions 31–34 (20 mg) showed no major component. Fractions 21–30 were combined, concentrated under reduced pressure, and rechromatographed on a preparative plate (alumina F-254, benzene-acetone-ammonia, 10:10:0.5) to give one major component and a small amount of a fluorescent band. Excision of the major band and extraction with chloroform-methanol (95:5) gave laurelliptine (**9**), the main component, as a glass (10.4 mg); $[\alpha]^{25}D$ +34° (c=0.5, MeOH); uv λmax (MeOH) 304, 284 and 220 nm (ε 6300, 5800 and 1500); mass spectrum *m/e* 313 (M^+, 80), 312 (100), 298 (17), 284 (15), 282 (7), 269 (7), 254 (7) and 253 (11). This compound gave positive tests with Dragendorff and ferric chloride reagents. Mixed tlc (silica gel) of **9** and laurelliptine in two solvent systems (acetone-methanol, 3:2; chloroform-methanol 17:3) demonstrated their identity. The mass spectra of laurelliptine and our alkaloid, run consecutively, were identical.

SELECTIVE *N*-ACYLATION-*O*-TRIMETHYLSILYLATION OF **9**.—Compound **9** (1 mg) in ethyl acetate was treated with BSTFA (30 μl) and heated at 80° for 1 hr. Heptafluorobutyric anhydride (HFBA) (10 μl) was added to the trimethylsilyl derivative, and the reaction mixture was allowed to stand for 1 hr at room temperature. The product was submitted immediately to mass spectral analysis; the spectrum showed *m/e* 653 (M^+, 100), 623 (12), 593 (3), 428 (21), 397 (40), 380 (7), 367 (22), 213 (7); calcd. for $C_{25}H_{34}NO_5Si_2F_7$, 653.

CONSTITUENTS OF THE LIGHT PETROLEUM FRACTION (C).—This fraction (106.9 g) was dissolved in ether (1 liter) and extracted (3 x 1 liter) with Na_2CO_3 solution (5%). It was then washed with distilled water (3 x 1 liter) and dried over anhydrous sodium sulfate, filtered and concentrated to give a green oily neutral fraction (91.5 g). The alkaline extracts were neutralized with hydrochloric acid and extracted with ether; the ether extract was washed with water, dried, and concentrated to give an oily acidic fraction (0.86 g).

1. *Neutral Components.* The neutral fraction (15 g) was chromatographed on an aluminum oxide column (Woelm, activity III, 500 g) with gradient elution with hexane, hexane-benzene (95:5, 9:1, 3:1 and 1:1), benzene, benzene-chloroform (98:2, 95:5, 9:1), chloroform, chloroform-methanol (99:1, 98:2, 95:5, 9:1, 3:1 and 1:1), and methanol. Fractions eluted with hexane and hexane-benzene (95:5, 9:1) yielded mixtures of hydrocarbons; ir (film) 2910, 2850 and 1460

cm^{-1}, which, after crystallization from hexane, furnished colorless crystals (70 mg) of $C_{30}H_{62}$, mp 54–56°; ir (film) identical to that above; ^1H nmr ($CDCl_3$) δ 0.9, 1.3; mass spectrum *m/e* 422 (M^+, 5), 393 (30), 365 (52), 337 (40), 125 (100). Fractions eluted with hexane-benzene (3:1, 1:1) and benzene, gave mixtures of alkyl alkanoate esters: ir (film) 2960, 2880, 1730, 1450 cm^{-1}, and β-sitosterol (120 mg) crystallized from ether-methanol as colorless plates, mp 135–138° (lit. (21) mp 140°); Liebermann-Burchard reaction, blue; ir (film) 3400, 1660, 1050, 830 and 780 cm^{-1}; ^1H nmr ($CDCl_3$) δ 1.0–0.7 (18H, m), 1.6 (s, OH, disappeared after exchange with D_2O), 5.4 (1H, m, –C=CH–); mass spectrum *m/e* 414 (M^+, 100), 399 (33), 396 (68), 381 (35), 255 (55), 231 (31), 213 (51), 159 (48), 119 (37). The mixed melting point and co-tlc (silica gel, hexane-benzene, 1:1) with an authentic sample confirmed the identity.

Fractions eluted with chloroform were further purified by rechromatography on silica gel (activity III) with toluene-ethyl acetate (95:5) to give an additional quantity of β-sitosterol (40 mg) and mixtures of unsaturated alkyl alcohols; ir (film) 3500, 3030, 2950, 2880 and 1450 cm^{-1}; ^1H nmr ($CDCl_3$) δ 5.4 (m), 4.2 (d, J=6 Hz), 2.0 (m), 1.7 (s), 1.3 (br, s), 0.9 (s), 0.8 (s). The remaining fractions did not yield characterizable compounds and were not investigated further.

2. *Acidic Compounds.* The acidic material from fraction (C) (0.86 g) was chromatographed on a silica gel column (Woelm, activity III, 30 g) employing gradient elution *via* hexane and hexane-ethyl acetate mixtures (99:1, 98:2, 95:5, 9:1, 3:1 and 1:1), ethyl acetate, and methanol. The hexane-ethyl acetate eluates (95:5, 9:1) furnished a mixture of carboxylic acids; ir (film) 3500–2600 (broad), 1710 cm^{-1}. The acids (3 mg) were methylated with diazomethane, and the esters (ir (film) 2960, 2880, 1730 cm^{-1}) were analyzed by gas-liquid chromatography on an SE-30 column equipped with a flame ionization detector. The column temperature was maintained at 210°; the carrier gas was N_2 (100 ml/min). The glc analysis indicated a mixture of at least seven components in the ester product.

The hexane-ethyl acetate eluate (3:1) gave an additional amount of carboxylic acids, ir (film) 3500–2700 (broad), 1710 cm^{-1}. Methylation of this mixture and glc analysis as described above indicated a mixture of five components.

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