*Helvolic acid.* 40 Megacycles per second, solvent, deuterochloroform; reference, external chloroform. $\tau$ values in ppm. for peaks: 2.48 (s), 3.05 (m), 4.01 (s), 4.25 (s), 4.77 (m), 7.64 (s), 7.90 (l), 8.08 (l), 8.34 (l), 8.39 (l), 8.56 (l), 8.68 (m), 8.83 (l), 9.10 (l).

*Methyl helvolate.* (See Fig. 1) 40 Megacycles; solvent, deuterochloroform; reference, external methylene chloride. $\tau$ values in ppm. for ppm. for peaks: 2.50 (s), 2.74 (s), 4.01 (s), 4.27 (s), 4.77 (m), 6.41 (l), 7.67 (s), 7.93 (l), 8.08 (l), 8.38 (l), 8.43 (l), 8.60 (l), 8.72 (m), 8.87 (l), 9.14 (l).

*Methyl tetrahydrohelvolate.* 60 Megacycles; solvent, deuterochloroform; reference, internal tetramethyl silane. $\tau$ values in ppm. for peaks: 4.09 (s), 4.20 (s), 4.71 (m), 6.33 (l), 7.56 (m), 7.89 (l), 8.02 (l), 8.68 (l), 8.77 (m), 8.88 (m), 9.07 (l), 9.16 (m).

*Octahydrohelvolic acid.* 60 Megacycles; solvent, deuterochloroform; reference, internal tetramethyl silane. $\tau$ values in p.p.m. for peaks: 4.21 (l), 4.72 (m), 7.92 (l), 7.99 (l), 8.45 (m), 8.77 (l), 9.09 (l), 9.19 (l).

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(CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, ANDHRA UNIVERSITY)

# Chemical Examination of *Embelia ribes*. I. Isolation of a New Constituent, "Vilangin," Its Constitution and Synthesis

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A new constituent, "vilangin" (I) has been isolated from the dry ripe berries of *Embelia ribes*. From a study of its reactions and degradation products, vilangin has been assigned the structure, methylenebis(2,5-dihydroxy-4-undecyl-3,6-benzoquinone). This has been confirmed by synthesis using embelin (XVII) and formaldehyde.

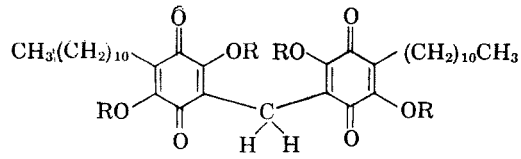
The dry berries of *Embelia ribes* are extensively used in India on account of their anthelmintic and antibiotic properties.[1] The active principle, so far isolated and extensively studied, is embelin.[2] Its constitution and synthesis have also been reported.[2] In all these experiments, the authors used ether, ethyl alcohol, benzene, and ethyl acetate as solvents for extraction. When purified methyl alcohol was used for extraction, besides embelin, a small quantity of a new entity melting at 264–265° was obtained. When methyl alcohol was replaced by purified dioxane, the yield of the new component considerably increased. The new substance is designated by us as "vilangin," a name taken from the vernacular Telugu name Vayuvilanga for *Embelia ribes*. We report in this communication, the isolation, constitution and synthesis of vilangin.

Vilangin is bright orange-yellow in color and is insoluble or sparingly soluble in common organic solvents, but easily soluble in dioxane and nitrobenzene. This low solubility and its occurrence in low yield in the natural berries, contributed largely to the failure of its isolation by earlier workers. It exhibited acidic properties by dissolving in alcoholic sodium, potassium and ammonium hydroxides, forming violet, deep violet, and pale violet solutions from which vilangin was regenerated by acidification. From aqueous alcoholic solutions of the alkalis, however, the corresponding salts were precipitated in crystalline condition. Vilangin also dissolved very slowly in aqueous alcoholic solutions of bicarbonate and carbonate with effervescence. With boric acid in concentrated sulfuric acid solution, it gave an intense green fluorescence under the ultraviolet lamp.

Vilangin gave an analysis corresponding to $C_{35}H_{52}O_8$, a formula confirmed by a Rast molecular weight of 610. It contained no alkoxyl groups, and since it was not cleaved when boiled with hydriodic acid, it was presumed to contain no oxide linkages. Its acidic character and an intense brown color formed with ferric chloride in dioxane indicated the presence of phenolic hydroxyls. On heating with orthophosphoric acid or boiling with concentrated sulfuric acid in dioxane solution, anhydrovilangin (II) was obtained, indicative of two hydroxyl

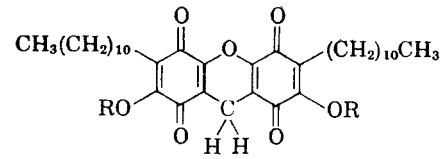
(1) G. H. Harris, *Lancet*, [2], 199 (1887); L. Scott, *Chemist and Druggist*, 163 (1888); C. J. H. Warden, *Pharm. J.*, 18, [3], 601 (1888); A. S. Paranjpé and Gopala Krishna Gokhalé, *Arch. Int. Pharmacodyn. Therap.*, 42, 212 (1932); Mariam George and K. M. Pandalai, *J. Indian Med. Res.*, 169 (1949); *U. S. Dispensatory*, 24th edit. 1950, p. 1441; *Glossary of Indian Medicinal Plants*, R. N. Chopra, S. L. Nayar, and I. C. Chopra, 1956, p. 106.

(2) C. J. H. Warden, *Pharm. J.*, 305 (1888); A. Heffter and W. Feurstein, *Arch. Pharm. Berlin*, 238, 15 (1900); R. Kaul, A. C. Ray, and S. Dutt, *J. Indian Chem. Soc.*, 6, 577 (1929); 8, 231 (1931); K. S. Nargund and B. W. Bhide, *J. Indian Chem. Soc.*, 8, 237 (1931); M. Asano and K. Yamaguti, *J. Pharm. Soc., Japan*, 60, 105 (1940); M. Asano and K. Yamaguti, *Proc. Imp. Acad., Japan*, 16, 36 (1940); M. Asano and Z. Hase, *J. Pharm. Soc. Japan*, 60, 650 (1940); L. F. Fieser and E. M. Chamberlin, *J. Am. Chem. Soc.*, 70, 71 (1948); V. Venkateswarlu and Ch. Bheemasankara Rao, *Current Sci. (India)*, 29, 136 (1960).

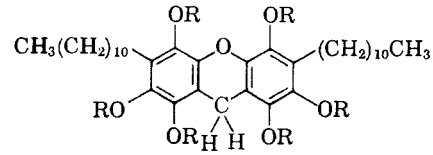


I. R = H
V. R = —$CH_3$
III. R = —$COCH_3$
VI. R = —$COC_6H_5$

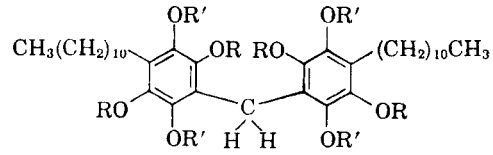
groups which could be dehydrated readily. This (II) could be converted back to vilangin by warming with dilute potassium hydroxide, and gave on acetylation di-*O*-acetylanhydrovilangin (IV) and on reductive acetylation, hexa-*O*-acetyltetrahydroanhydrovilangin (VIII). The formation of these anhydrides along with the normal derivatives was also noticed in the preparation of some of the derivatives of vilangin. On acetylation using acetic anhydride and pyridine, vilangin gave two acetates: (1) an orange-yellow tetra-*O*-acetylvilangin (III) and (2) a reddish di-*O*-acetylanhydrovilangin (IV). Vilangin could be recovered from the former acetate (III) by hydrolysis, while the latter (IV) on hydrolysis gave anhydrovilangin (II) identical with the product (II) obtained by the dehydration of vilangin (I). Methylation of vilangin (I) using excess diazomethane in dioxane-ether solution gave yellow tetra-*O*-methylvilangin (V) and on benzoylation, pale yellow tetra-*O*-benzoylvilangin (VI). These reactions indicated the existence of four phenolic hydroxyl groups. Further it showed no positive evidence of any alcoholic hydroxyl groups as tetra-*O*-methylvilangin (V) did not respond to acetylation. Reductive acetylation of vilangin gave two leuco acetates: (1) octa-*O*-acetyltetrahydrovilangin (VII) from which vilangin could be recovered by alkali hydrolysis followed by acidification and aerial oxidation; (2) hexa-*O*-acetyltetrahydroanhydrovilangin (VIII), which on treatment with alkali gave anhydrovilangin (II). Reduction of vilangin by the Clemmensen method gave tetrahydrovilangin (IX), which on acetylation gave octa-*O*-acetyltetrahydrovilangin (VII), identical with the sample obtained earlier by the reductive acetylation of vilangin. Tetra-*O*-methylvilangin (V) could further be reduced using sulfur dioxide in methanol solution to give tetra-*O*-methyl-tetrahydrovilangin (X) which in turn gave tetra-*O*-methyl-tetra-O-acetyl-tetrahydrovilangin (XI). The same could directly be obtained by the reductive acetylation of tetra-*O*-methylvilangin (V). These reactions indicated the existence of at least two quinone units which could easily be reduced and reoxidized. This observation of the existence of two quinone units is further confirmed by the ready formation of (1) a tetroxime (XII), (2) a 2,4-dinitrophenylhydrazone (XIII), (3) a tetraanil (XIV) and (4) a tetradesoxytetramethylimino compounds. Further, oxidation of vilangin using alkaline permanganate gave *n*-lauric acid, in addition to lower aliphatic carboxylic acids, whilst



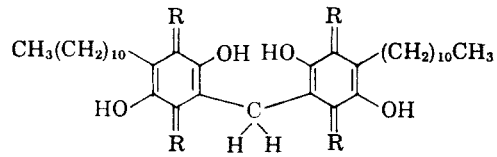
II. R = H
IV. R = $COCH_3$



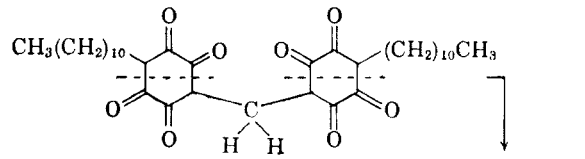
VIII. R = —$COCH_3$



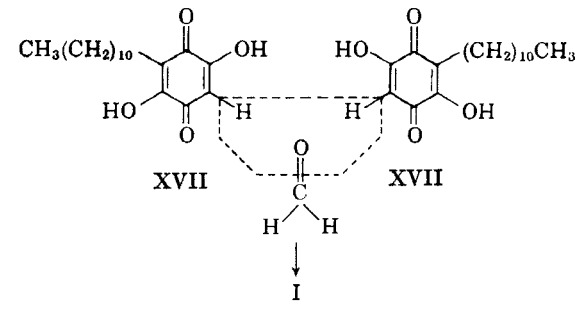
VII. R = R′ = —$COCH_3$
IX. R = R′ = H
X. R = —$CH_3$; R′ = H
XI. R = —$CH_3$; R′ = —$COCH_3$



XII. R = =NOH
XIII. R = =$NNHC_6H_3(NO_2)_2$
XIV. R = =$NC_6H_5$
XV. R = =$NCH_3$



2 $CH_3(CH_2)_{10}CH_2COCOOH$
XVI



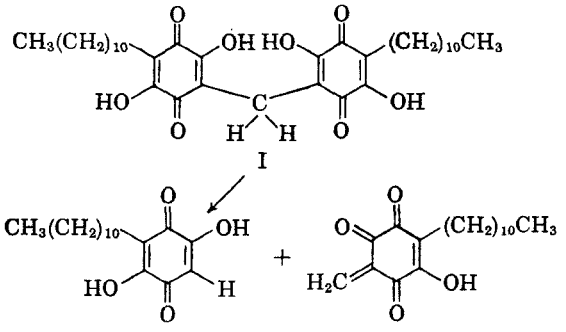
XVII XVII

I

alkali fission of vilangin gave α-ketomyristic acid (XVI) as the main product. Both *n*-lauric acid and α-ketomyristic acid were products of oxidative degradation and alkali hydrolysis of embelin[2] (XVII) respectively. These observations were fur-

ther supported by the isolation of embelin by the pyrolysis of vilangin. The constitution of vilangin (I) should therefore be represented as two hydroxylated benzoquinone units with side chain $-(CH_2)_{10}-CH_3$, bridged through a methylene group as in (I).

That structure I correctly represents vilangin was proven by an unambiguous synthesis from embelin (XVII). Embelin condensed with formaldehyde in acetic acid solution to give methylenebis-(2,5-dihydroxy-4-undecyl-3,6-benzoquinone), identical with natural vilangin. The formation of embelin from vilangin could easily be explained by the following scheme:



I

XVII XVIII

While embelin (XVII) could undergo sublimation, the second part (XVIII) could be expected either to decompose or polymerize.

Although other dibenzoquinones are known, *e.g.* phoenicin and oosperein,[3] vilangin seems to be the first known example of a methyl-bisbenzoquinone, the formation of which could take place readily in the plant.

Vilangin was found to occur in the dry berries of *Embelia robusta* also, up to 0.06% along with embelin (3%).

## EXPERIMENTAL

*Isolation of vilangin* (I). Dry ripe berries of *Embelia ribes* were crushed to a coarse powder (1 kg.) and extracted in a Soxhlet extracter with purified dioxane until the fresh extract was no longer colored. The extract was then concentrated under reduced pressure and the solids that had separated on refrigeration were filtered and the filtrates diluted with water. The solids that had separated were added to the main bulk, dried and macerated with cold petroleum ether (b.p. 40–60°) and filtered. The solid residues were then separated into ether-soluble (A, 30 g.) and ether-insoluble (B, 1 g.) fractions. The ether-soluble fraction (A) gave crude embelin which on crystallization from benzene and finally from alcohol appeared as orange lustrous plates melting at 143°, identical with the product isolated using ether as solvent.

*Anal.* Calcd. for $C_{17}H_{26}O_4$: C, 69.34; H, 8.91. Found: C, 69.52; H, 9.04.

The ether-insoluble fraction (B) appeared as brownish yellow crystals insoluble in ether, acetone, methanol, ethanol, chloroform, carbon tetrachloride, *etc.* Recrystallized from warm dioxane or nitrobenzene, it appeared as small bright orange yellow prisms melting at 264–265° dec. (yield pure: 750 mg.).

*Anal.* Calcd. for $C_{35}H_{52}O_8$: C, 69.93; H, 8.73. Found: C, 70.12, 70.24; H, 8.83, 9.02.

Vilangin dissolved in alcoholic sodium hydroxide forming a greyish violet solution. With alcoholic potassium hydroxide, deep violet, and with alcoholic ammonium hydroxide, pale violet solutions were formed from which vilangin could be recovered by acidification. The alkali salts were, however, found to be sparingly soluble in water. Vilangin dissolved with difficulty in aqueous alcoholic sodium carbonate or bicarbonate with effervescence. In pyridine or piperidine, it dissolved quite readily, forming an intense red solution from which vilangin could be recovered by treatment with excess dilute hydrochloric acid. With ferric chloride in dioxane solution, vilangin gave a deep brown color. In concentrated sulfuric acid, it dissolved only on warming and was not regenerated on dilution with water. With boric acid in concentrated sulfuric acid, vilangin gave an intense green fluorescence under ultraviolet light, while the solution was colored pale yellow. On sublimation at high temperature (240–245°/0.001 mm.), vilangin underwent easy sublimation and the sublimed sample had the same melting point; while at lower temperature (205–210°/0.001 mm.), it was observed to undergo slow pyrolysis and the sublimed sample melted at 143° and was identified as embelin.

Its *2,4-dinitrophenylhydrazone* (XIII), appeared as deep red rhombs and did not melt below 320°.

*Anal.* Calcd. for $C_{59}H_{68}O_{20}N_{16}$: N, 16.96. Found: N, 16.74.

Its *tetroxime* (XII) appeared as deep yellow prisms, m.p. 128–29°.

*Anal.* Calcd. for $C_{35}H_{56}O_8N_4$: N, 8.51. Found: N, 8.63.

Condensation of I with excess methylamine gave *tetradesoxytetramethyliminovilangin* (XV) as deep violet short prisms, m.p. 115–17° dec. (from ethyl acetate-petroleum ether, b.p. 40–60°) with a brown ferric reaction.

*Anal.* Calcd. for $C_{39}H_{64}O_4N_4$: N, 8.58. Found: N, 8.81.

Its *acetate*, (tetraacetate of XV) appeared as colorless prisms from methanol and melted at 127–129°.

With aniline, I condensed quite readily in presence of 2 drops of concd. sulfuric acid, giving *vilangin tetraanil* (XIV), obtained as green rectangular flakes from benzene, m.p. 188–189°. It gave an intense green color with alcoholic ferric chloride, while the solution was colored pale yellowish green.

*Anal.* Calcd. for $C_{59}H_{72}O_4N_4$: N, 6.21. Found: N, 6.52.

*Tetrahydrovilangin* (IX). Reduction of I in dioxane solution following the Clemmensen method using zinc amalgam and hydrochloric acid gave IX as colorless prisms from methanol, m.p. 243–245° dec.

*Anal.* Calcd. for $C_{35}H_{56}O_8$: C, 69.49; H, 9.33. Found: C, 69.53; H, 9.52.

It dissolved readily in 2*N* sodium hydroxide and gave a deep red-brown color changing to black with neutral ferric chloride in alcoholic solution.

*Anhydrovilangin* (II). II was obtained by boiling I with (a) 20% sulfuric acid in dioxane solution during 3 hr. or (b) 30% orthophosphoric acid in dioxane solution during 1 hr. In both cases, II was obtained as brown prisms from petroleum ether (b.p. 60–80°), m.p. 116–118°.

*Anal.* Calcd. for $C_{35}H_{50}O_7$: C, 72.11; H, 8.65. Found: C, 72.61; H, 9.04.

It was soluble in aqueous alkali and gave a red-brown ferric color in alcoholic solution. When the dehydration was done using hydriodic acid in acetic anhydride by boiling during 1 hr., a mixture of I and II was obtained which was separated using benzene in which I was insoluble. When acetic anhydride was replaced by phenol, the dehydration was complete and II was the only entity recovered.

Acetylation of II using acetic anhydride and a drop of sulfuric acid gave *di-O-acetylanhydrovilangin* (IV) and re-

(3) T. Posternak, *Helv. Chim. Acta*, **21**, 1326 (1938); T. Posternak, H. W. Ruelius, and J. Tcherniak, *Helv. Chim. Acta*, **26**, 2031 (1943); F. Kögl and G. C. van Wessem, *Rec. trav. chim.*, Pays-Bas, **63**, 5 (1944); G. Lloyd, A. Robertson, G. B. Sankey, and W. B. Whalley, *J. Chem. Soc.*, 2163 (1955).

ductive acetylation gave *hexa-O-acetyltetrahydroanhydrovilangin* (VIII).

II was converted back to I by digestion with 8*N* potassium hydroxide on a steam bath for 3 hr.

*Tetra-O-benzoylvilangin* (VI) was obtained by the benzoylation of I in pyridine solution using benzoyl chloride, as pale yellow prisms (from ethyl acetate-petroleum ether, b.p. 40–60°), m.p. 174–175°.

*Anal.* Calcd. for $C_{62}H_{68}O_{12}$: C, 74.35; H, 6.74. Found: C, 74.64; H, 7.03.

It was insoluble in aqueous alkali and gave no positive ferric reaction.

*Tetra-O-methylvilangin* (V) was obtained by the methylation of I (0.5 g.) in dioxane solution (20 ml.) using excess diazomethane. Crystallized from methanol, V appeared as pale yellow rectangular prisms, m.p. 86–87°, having no positive ferric reaction; yield, 0.4 g.

*Anal.* Calcd. for $C_{39}H_{60}O_8$: C, 71.29; H, 9.22; —$OCH_3$, 18.90. Found: C, 71.62; H, 9.41; —$OCH_3$, 19.23.

Its *2,4-dinitrophenylhydrazone* appeared as long red-brown needles (from methanol), m.p. 120–22°.

*Anal.* Calcd. for $C_{63}H_{76}O_{20}N_{16}$: N, 16.65. Found: N, 17.42.

Reduction of V in methanol (200 mg. in 50 ml.) using sulfur dioxide gave *tetra-O-methyltetrahydrovilangin* (X) as colorless needles from alcohol, m.p. 155–157°; yield, 150 mg.

*Anal.* Calcd. for $C_{39}H_{64}O_8$: C, 70.86; H, 9.77. Found: C, 71.22; H, 10.24.

It dissolved readily in aqueous alkali and gave no prominent ferric color in alcoholic solution. Its *acetate*, (XI, prepared from X), melted at 94–95° and was found to be identical with the sample obtained directly by the reductive acetylation of V.

Reductive acetylation of V (250 mg.) using zinc dust in acetic anhydride (4 ml.) in presence of a drop of triethylamine gave XI as colorless prisms from methanol, m.p. 94–95°; yield, 150 mg.

*Anal.* Calcd. for $C_{47}H_{72}O_{12}$: C, 68.08; H, 8.76. Found: C, 68.33; H, 8.94.

It was insoluble in dilute alkali and gave a negative ferric reaction.

*Acetylation of vilangin* (I). *Isolation of* (III) *and* (IV). Acetylation of I (1 g.) using acetic anhydride (15 ml.) in presence of pyridine, gave a mixture of two acetates separated by fractional crystallization using acetone–petroleum ether (b.p. 40–60°): (1) sparingly soluble fraction (III) which appeared as orange yellow prisms (yield: 350 mg.) from ethyl acetate-petroleum ether, m.p. 174–76°, which on hydrolysis using cold alkali (8*N*) gave I. A mixed melting point with natural vilangin was undepressed.

*Anal.* Calcd. for $C_{43}H_{60}O_{12}$: C, 67.13; H, 7.87; —$COCH_3$, 22.39. Found: C, 67.42; H, 8.03; —$COCH_3$, 22.64.

The second acetate was obtained as orange-red prisms (IV), (150 mg.), m.p. 110–12°, which on hydrolysis gave a product, identified as II by a mixed melting point with the sample obtained earlier.

*Anal.* Calcd. for $C_{39}H_{54}O_9$: C, 70.20; H, 7.84; —$COCH_3$, 13.12. Found: C, 70.43; H, 8.02; —$COCH_3$, 13.54.

*Reductive acetylation of* I. *Isolation of octa-O-acetyltetrahydrovilangin* (VII) *and hexa-O-acetyltetrahydroanhydrovilangin* (VIII). Digestion of I (1 g.) in acetic anhydride (25 ml.) in the presence of zinc dust (3 g.) and a trace of triethylamine during 2 hr. gave a mixture of two substances, m.p. 205–212°, separated by fractional crystallization using acetic acid into (a) acetic acid sparingly soluble fraction (VIII, 0.4 g.), as colorless prisms (from acetic acid), m.p. 224–225°, (hydrolysis of VIII using 8*N* alkali followed by acidification and aerial oxidation gave II).

*Anal.* Calcd. for $C_{47}H_{66}O_{13}$: C, 67.26; H, 7.93; —$COCH_3$, 20.52. Found: C, 67.54; H, 8.02; —$COCH_3$, 20.83.

(b) An acetic acid-soluble fraction (VII) was obtained as clusters of colorless short prisms (250 mg.), m.p. 170–171° from methanol, which on hydrolysis with alkali and acidification gave I, a mixed melting point with vilangin being undepressed.

*Anal.* Calcd. for $C_{51}H_{72}O_{16}$: C, 65.07; H, 7.72; —$COCH_3$, 36.60. Found: C, 65.31; H, 8.04; —$COCH_3$, 37.01.

*Oxidation of I using potassium permanganate. Isolation of n-lauric acid.* Oxidation of (I) (2.5 g.) in dioxane (50 ml.) using potassium permanganate (3 g.) gave a mixture of vilangin and a colorless acid, which could be separated using ether as solvent. Crystallized from alcohol, the acid appeared as colorless plates and prisms, m.p. 42–43°, identified as *n*-lauric acid by a mixed melting point.

*Anal.* Calcd. for $C_{12}H_{24}O_2$: C, 71.94; H, 12.09. Found: C, 72.32; H, 12.10.

*Alkali fission of vilangin* (I). *Isolation of α-ketomyristic acid* (XVI). I was gently boiled under reflux on a steam bath for 12 hr. after the addition of aqueous alcoholic sodium hydroxide (10%, 100 ml. for each gram). On working up the reaction mixture, XVI was obtained as colorless plates, m.p. 63–64°, identified as *α*-ketomyristic acid by comparison with an authentic sample obtained by similar hydrolysis using embelin.

*Anal.* Calcd. for $C_{14}H_{26}O_3$: C, 69.36; H, 10.81. Found: C, 69.50; H, 10.92.

Further oxidation of XVI with alkaline potassium permanganate gave tridecanoic acid, m.p. 44–45° (from alcohol), identified further by the preparation of its zinc salt, m.p. 127–128°.

*Methylenebis(2,5 - dihydroxy - 4 - undecyl - 3,6 - benzoquinone)* (I). Embelin (1 g.) in warm acetic acid (30 ml.) was treated with a solution of formaldehyde (5 ml. 40%) and the resulting solution gently warmed on a steam bath for 10 min. The copious precipitate on crystallization from dioxane, appeared as bright orange-yellow prisms, insoluble in ether. A mixed melting point with natural vilangin was undepressed; yield, quantitative.

*Anal.* Calcd. for $C_{25}H_{52}O_8$: C, 69.93; H, 8.73. Found: C, 70.10; H, 8.82.

I was also obtained when the condensation was carried out in neutral or in alkaline solutions. However, when dihydroembelin was used instead of embelin, tetrahydrovilangin (IX) was obtained.

WALTAIR, INDIA