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THE STRUCTURE OF CARINDONE*†

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Abstract—Various spectroscopic and chemical methods have been employed to derive the structure of carindone as a novel type of C_{31}-terpenoid.

INTRODUCTION

THE CHEMICAL investigations on *Carissa carandas*[1,2] had led to the isolation of several substances including d-sitosterol, a mixture of cardenolides, carissone and a new substance, carindone. Studies undertaken to elucidate the constitution of the latter are reported in the present communication.

RESULTS AND DISCUSSION

Carindone was insoluble in sodium carbonate solution but readily dissolved in dilute sodium hydroxide from which it was recovered on acidification. It did not respond to characteristic colour reactions of phenols, flavonoids, triterpenoids and glycosides. It did not show any colour with tetranitromethane but gave a red colour in the Liebermann–Burchard test. With diazotized sulphanilic acid and sodium carbonate, it gave a bright canary yellow colour and this reagent has been used for its detection on TLC plates.

It analysed for $C_{31}H_{44}O_6$ which was confirmed by its accurate molecular weight determination by mass spectrometry. The UV spectrum of carindone showed absorption at 214, 251 and 297 nm (log ϵ 4·40, 4·31, 3·90 respectively). In alkali, the 214 and 297 nm peaks showed bathochromic shift to 229 and 336 nm respectively which strongly suggested the presence of α,β-unsaturated carbonyl and enolic type of system in the molecule. It showed pK_a of 8·96 in 50% alcohol. The IR spectrum of carindone showed hydroxyl functions, an α,β-unsaturated carbonyl and a β-diketo system in the molecule.

In the NMR spectrum of carindone there was a sharp 12 proton singlet at 1·26 ppm for four methyls of the type $-OC(CH_3)-$, two allylic methyls at 1·31 and 1·40 ppm and a 6-protons singlet at 1·95 ppm due to two methyls of the type $-COC(CH_3)=C$. The 1·20–2·50 ppm region, in addition to the methyl protons mentioned above, showed a broad hump of 16 protons which constituted the methylene protons of the molecule. The residual 4 protons appear in the region 2·80–3·40 ppm distributed as a broad 2 protons doublet at 2·86 and 3·08 ppm (J = 14 Hz) and another sharp two proton doublet centred at 3·33 ppm (J = 2 Hz). The NMR clearly indicate the presence of 8 methyls along with their chemical environment in the molecule. In the NMR spectrum taken in D_2O/NaOD, the two allylic methyls move upfield and appear as singlet at 1·16 ppm.

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† Part III in the series "Studies on *Carissa carandas*".

[1] R. C. RASTOGI, M. M. VOHRA, R. P. RASTOGI and M. L. DHAR, *Indian J. Chem.* **4**, 132 (1966).

[2] R. C. RASTOGI, R. P. RASTOGI and M. L. DHAR, *Indian J. Chem.* **5**, 215 (1967).

The acetylation of carindone with acetyl chloride, as described for chelated hydroxyls,[3] gave crystalline diacetate (M^+ at *m/e* 596) which did not show any IR absorption in the hydroxyl region but a strong band at 1740 cm^{-1} due to acetyl groups. The other bands of the original product maintained their respective positions with slight variation. The NMR spectrum showed 6 proton singlet for two –OAc functions at 2·00 ppm. The 12 proton singlet of 4 methyls was found to be shifted downfield to 1·50 ppm which confirmed the environment of these methyls as $-C-C(CH_3)_2-OAc$. The other four methyls (1·24, 1·300 and 1·95 (two) ppm) and four protons in the region 2·80–3·40 ppm remained unaffected.

The presence of only two tertiary hydroxyls in the molecule was further confirmed by the NMR spectrum of trichloroacetyl isocyanate (TAI) derivative of carindone which showed two N–H protons at 8·83 ppm. The 12 proton singlet was found to be shifted to 1·65 ppm and the two tertiary methyls were present at 1·36 and 1·45 ppm. The remaining two methyls of the type $-COC(CH_3)=C-$ were present at 1·96 ppm as a singlet. A broad doublet at 2·95 and 3·06 ppm of two protons (J = 10 Hz) and two protons at 3·35 ppm as a broad singlet were also present.

Acetylation of carindone with acetic anhydride–pyridine at room temperature led to the formation of a resinous product, more polar than the starting material which could not be purified and only turned into a brown gummy mass during a course of a few days. However, when the reaction mixture was worked up after 12 hr and the residue was immediately purified by preparative TLC, a major component was obtained which showed characteristic changes in IR spectrum. Two very strong absorption bands for enol acetate grouping appeared at 1775 and 1182 cm^{-1} with the simultaneous disappearance of the equally strong 1568 and 1550 cm^{-1} absorptions. This showed that carindone contains a keto system which under the reaction conditions enolizes with the formation of enol acetate derivative. However, acetylation with acetic anhydride under reductive conditions[4] led to the formation of carindone diacetate in poor yield, identical with the product obtained above.

Carindone was resistant to catalytic reduction with Adam's catalyst in ethanol or acetic acid indicating that the double bonds are either tetrasubstituted or hindered. It was recovered unchanged during lithium aluminium hydride and sodium borohydride reductions and did not yield any carbonyl derivative with 2,4-dinitrophenyl hydrazine or hydroxylamine.

Sodium–liquid ammonia reduction of carindone afforded a crystalline product, hydrocarindol, which did not show any absorption in UV and showed a very strong and broad hydroxyl band at 3350 cm^{-1}. The IR spectrum was characteristic by the absence of the carbonyl or unsaturation bands in the 1600–1700 cm^{-1} region. An outstanding feature of the NMR spectrum was that four methyls now appeared in the region 0·85–1·00 ppm, two of which gave singlets (0·85 and 0·90 ppm) while the remaining two methyls showed splitting and must be secondary in nature. The 12 proton singlet was present at 1·18 ppm and assigned to two $HO-C(CH_3)_2-$ groups. The 2 protons doublet at 3·33 ppm of the original compound disappeared as it moved up into the methylene envelope. The upward shift of the four methyls and the pattern of splitting in this region confirmed that in carindone the disposition of these would be—two as vinylic and the other two as quaternary and allylic in nature ($-COC(CH_3)=CC(CH_3)-$).

Acetylation of hydrocarindol gave a complex mixture consisting of 6 substances. Two of these components having lower R_f were separated as amorphous powders but could not be further purified because of insufficiency of the material. The NMR spectra of these

[3] S. SHIBATA, E. MORISHITA, T. TAKEDA and K. SAKATA, *Tetrahedron Letters* 4855 (1966).
[4] J. CORREA and J. ROMO, *Tetrahedron* **22**, 685 (1966).

derivatives, however, showed that these were di- and triacetyl derivatives in which the two $-C(OH)-(CH_3)_2$ groups were not affected. Hydrocarindol would, on this basis, contain 5 (possibly 6) hydroxyl groups.

Carindone did not react with sodium periodate and its oxidation with various reagents like alkaline $KMnO_4$ and CrO_3 in glacial acetic acid or pyridine were carried out. In each case intractable mixtures of coloured products were obtained which did not yield to any purification procedure.

Dehydrogenation of carindone with palladium–charcoal afforded two products A_1 and A_2 in very low yields. The major substance A_1 was crystalline and showed IR bands at 1655 and 1600 cm^{-1} for conjugated carbonyl, 1570 cm^{-1} for β-diketo system and UV maxima at 245 nm (log ϵ 4·18) and 281 nm (log ϵ 3·70). The maxima at 281 shifted to 326 nm (log ϵ 3·92) in presence of alkali. In the MS, substance A_1 gave M^+ peak at *m/e* 232 corresponding to the molecular formula $C_{15}H_{20}O_2$. The NMR of this product showed three methyls at 1·40, 1·75 and 1·86 ppm and two olefinic protons at 4·78 ppm.

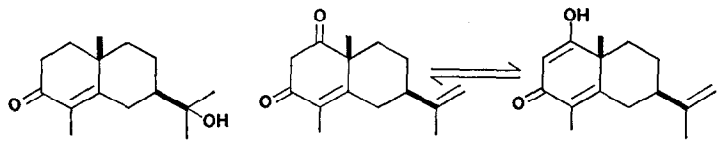
The MW of substance A_1 indicated that probably it arises by the splitting of the carindone molecule in the middle into two fragments. Further, its MS and MF ($C_{15}H_{20}O_2$) indicated its close similarity to carissone ($C_{15}H_{24}O_2$), a sesquiterpene (I) isolated in large amounts from this plant.[1] The mass spectrum of carissone has no molecular ion (M^+), but shows a peak at M^+-18 (*m/e* 218) which is also the base peak. The other fragments are *m/e* 203, 190, 175, 163, 147, 133, 122, 119, 105, 91, 71, 79, 67 and 59.

The IR spectrum of A_1 did not show any hydroxyl and its NMR was not indicative of $(CH_3)_2$–C(OH)– grouping which was also confirmed by the absence of *m/e* 59 peak $(CH_3)_2$–C=OH^+ in the MS of A_1 whereas carissone shows a strong ion peak at *m/e* 59. On the other hand, the IR, UV and NMR spectra of substance A_1 suggested that it contained a α,β-unsaturated carbonyl O=C–C(CH_3)=C– as well as a β-diketo system. The latter grouping is strongly supported by the loss of 28 mass units (CO) twice in the sequence shown in Fig. 1 and confirmed by the appropriate metastable (m*) peaks.

$$m/e\ 190 \xrightarrow{-CO} \underset{(m^*\ 138{\cdot}1)}{m/e\ 162} \xrightarrow{-CH3} \underset{(m^*\ 133{\cdot}4)}{m/e\ 147} \xrightarrow{-CO} \underset{(m^*\ 96{\cdot}3)}{m/e\ 119}$$

FIG. 1.

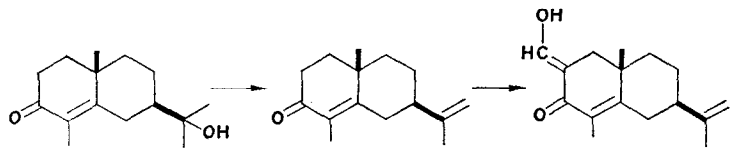
This data fixed the oxygenated chromophore in A_1 as –CO–C–CO–C(CH_3)=C–. Consequently, substance A_1 would be 'keto-anhydro carissone' (II).



(I) (II)

The above-mentioned data leads to the speculation that carindone is synthesized in the plant by condensation of two units of carissone and involving incorporation of one extra carbon and two oxygen atoms with simultaneous loss of 4 hydrogens.

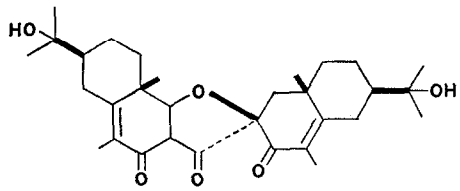
The C and O should be incorporated so as to give rise to a β-diketo system in the molecule. The loss of 4 hydrogens implies that each of the two precursor units must be linked at two positions to one another. Such a possibility is not unlikely because the plant elaborates



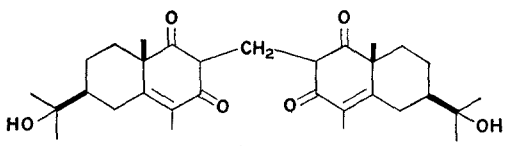
(I) Carrisone (III) α-Cyperone 2-Formyl-α-cyperone

large amounts of carissone (2%) and this dimerization may be a very minor offshoot in the biogenetic pathway of carissone or one of its precursors. Further, the additional carbon could have originated from the incorporation of a C_1 unit somewhere in the scheme. Chemically this feasibility has been shown by the easy formylation of α-cyperone[5] (III).

The formylated carissone (or its precursor) is expected readily to lend itself to dimerization or condensation with an appropriate precursor. Thus, two possible structures IV and V can be postulated for carindone. The NMR spectra of carindone in $CDCl_3$ and in $NaOD/D_2O$ indicate that IV is the most plausible and is, therefore, proposed to be the structure of carindone.



(IV)



(V)

EXPERIMENTAL

All the melting points are uncorrected. The NMR spectra were recorded on Varian A-60D in $CDCl_3$ with TMS as internal standard. The R_f values pertain to TLC on silica gel plates.

Carindone. m.p. 262°, λ_{max} (alcohol) 214, 251, 297 nm (log ε 4·40, 4·31, 3·90 respectively). λ_{max} (N/100 KOH) 229, 249, 336 nm (log ε 4·425, 4·21, 3·90 respectively). ν_{max} (KBr): 3475, 3400 (OH), 2615 (W and broad, OH intramol. H-bonded) 1647, 1595 (α,β-unsaturated CO), 1568, 1550 (β-diketone), 1385, 1370, 1183 (isopropyl), 825 (C=C), 1450, 1430, 1310, 1270, 1045, 1005, 910 cm^{-1}; NMR: ppm 1·26 (12H, s, 2.—C—$C(CH_3)_2OH$, 1·31, 1·40 (3H each, s, 2 allylic CH_3), 1·95 (6H, s, 2.—CO—$C(CH_3)$=C—), 2·86, 3·08 (2H, broad d, J = 14 Hz) and 3·33 (2H, d, J = 2 Hz); MW (by MS), 512·3132.

Acetylation of carindone. (i) Carindone (100 mg) in dry $CHCl_3$ (8 ml) solution was cooled, AcCl (3 ml) and pyridine (2·4 ml) was added to it and the mixture was allowed to stand overnight at 0°. Ice was added and the reaction mixture was worked up in the usual manner. The residue from the $CHCl_3$ extract was crystallized from MeOH as colourless needles, m.p. 217–219°, 86 mg, R_f 0·81 (benzene–MeOH 1·5%). ν_{max} (KBr): 1740 ($OCOCH_3$), 1660, 1601, 1590, 1570 (unsaturated CO and β-diketone), 1380, 1185 (isopropyl), 1435, 1400, 1340, 1250, 1048, 1030 and 845 cm^{-1}. NMR: ppm 2·00 (6H, s, 2·$OCOCH_3$), 1·50 (12H, s, 2 · –C–COH–$(CH_3)_2$–),1·24, 1·30 (3H each, s, allylic CH_3), 1·95 (6H, s, –CO–$C(CH_3)$=C–), 2·71–2·98 (2H, broad d, J = 10 Hz) and 3·34 (2H, d, J = 3 Hz). (Found: C, 70·24; H, 8·15; MW (MS) 596. $C_{33}H_{48}O_8$ requires: C, 70·46; H, 8·05%).

(ii) Carindone (100 mg), fused NaOAc (50 mg), Ac_2O anhydride (2 ml) and Zn powder (500 mg) were refluxed for 40 min. After working up, the residue was chromatographed over silica gel and benzene–$CHCl_3$ (1 : 1) eluate gave a residue which crystallized from MeOH, m.p. 217°, 13 mg.

Na-*liquid ammonia reduction of carindone.* Carindone (200 mg) was stirred with Na in liquid NH_3 at −70° for 3 hr in the usual manner. After the evaporation of NH_3 at room temperature, aqueous NH_4Cl was added, the resultant precipitate was filtered and washed (H_2O). The powder was chromatographed over alumina (activity 3) and EtOAc eluate gave a crystalline substance (hydro carindol), 180 mg, m.p. 280°, R_f 0·27 (benzene–MeOH 10%). ν_{max} (KBr): 3350 (strong, OH), 1470, 1440, 1380, 1190, 1085, 1030, 1010, 910 cm^{-1}. NMR ($CDCl_3$ and DMSO-d_6): ppm 0·85, 0·90 (3H each, s, $2CH_3$), 0·88–1·00 (6H, 2 sec. CH_3),

[5] A. E. BRADFIELD, B. H. HEGOLE, B. S. RAO, J. L. SIMONSEN and A. E. GILLAM, *J. Chem. Soc.* 667 (1936).
[6] H. HIKINO, K. AOTA and T. TAKEMOTO, *Chem. Pharm. Bull. Tokyo* **15**, 1929 (1967).

1·18 (12H, s, 2. C–C(CH_3)$_2$OH) and 2·7–3·2 (3H, m, CHOH). (Found: C, 68·90; H, 11·13. $C_{31}H_{54}O_6$ requires: C, 71·0; H, 10·3%.)

Acetylation of hydrocarindol. Hydrocarindol (100 mg) was treated with Ac_2O (2 ml) and pyridine (1 ml. overnight at room temperature. After the usual working up, the residue (95 mg) showed 3 major spots, two of which (i) R_f 0·22 and (iii) R_f 0·30 were obtained as amorphous powders by preparative TLC in benzene–MeOH (10%). (i) and (iii) ν_{max} (KBr): 1735 (strong, $OCOCH_3$) and 3350 cm^{-1} (weak, OH). (i) NMR: ppm 0·80–1·05 (12H, 4CH_3), 1·20 (12H, s, 2. –C–C(OH)(CH_3)$_2$), 2·12 (6H, s, 2 $OCOCH_3$) and 4·1–4·9 (2H, m, 2 CHOAc). (iii) NMR: ppm 0·80–1·05 (12H, 4 CH_3), 1·20 (12H, s, 2. –C–C(OH)(CH_3)$_2$), 2·08 (3H, s, $OCOCH_3$) and 2·10 (6H, s, 2 $OCOCH_3$) and 4·2–5·0 (3H, m, 3 CHOAc).

Dehydrogenation of carindone. Carindone (200 mg) was mixed with Pd/C (30%, 240 mg) and heated at 310° for 30 min in N_2. The mixture was extracted with $CHCl_3$ and the solvent layer was separated into acidic acid and neutral fractions by extraction with NaOH. The neutral fraction gave resinous material.

The alkaline layer was acidified, extracted with $CHCl_3$ and worked up as usual. The residue showed two spots—A_1, R_f 0·16 and A_2, R_f 0·26 in benzene–MeOH (2%) as bright yellow spots on spraying with diazotized sulphanilic acid. These were separated by preparative TLC (benzene–MeOH 4%), but substance A_2, 6 mg, could not be crystallized. Substance A_1 was obtained as colourless needles from methanol, m.p. 185–187°, 10 mg. λ_{max} (alcohol) 245, 281 nm (log ϵ 4·18, 3·70). λ_{max} (N/100 KOH) 246, 236 nm (log ϵ 3·99, 3·92). ν_{max} (KBr): 1645, 1600, 1570 (conjugated CO and β-diketone) 1655,885 (terminal methylene), 840 (trisubstituted C=C), 1510, 1495, 1395, 1375, 1320, 1300, 1250, 1230, 1200 and 855 cm^{-1}. NMR: ppm 1·40, 1·75, 1·86 (3H each, s, 3 CH_3), 4·78 (2H, olefinic H) and 3·35–4·0 (2H, broad). MS: 232 (M^+), 217, 214, 204, 190, 175, 162, 147 (base peak), 134, 122, 119, 105, 91, 79, 77, 67 and 53. MW (by MS) 232. MF $C_{15}H_{20}O_2$ calculated from M^+.

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Key Word Index—Carissa carandas; Apocynaceae; terpene; carindone.