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ISHWARANE AND ARISTOLOCHENE, TWO NEW SESQUITERPENE HYDROCARBONS FROM *ARISTOLOCHIA INDICA**

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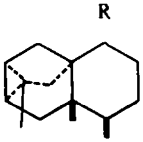
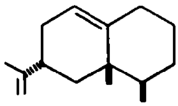
Abstract—From the roots of *Aristolochia indica*, two new sesquiterpene hydrocarbons, named ishwarane and aristolochene, have been isolated and assigned structures II and III respectively.

ISHWARONE,[1] one of the chief constituents of the roots of *Aristolochia indica* (Aristolochiaceae), has been recently formulated[2, 3] as I on the basis of convincing chemical and spectral evidence. In view of the unique structural features, we were prompted to examine the other constituents of *Aristolochia indica*, for sesquiterpenoids of biogenetic interest. We have isolated two new sesquiterpene hydrocarbons, designated ishwarane and aristolochene. The present paper describes the isolation and structural determination of these hydrocarbons as II and III respectively.



I : R = O
II : R = H_2



III

The oily residue from the hexane extract of the root was distilled *in vacuo* and the fraction collected below 110°/1 mm was chromatographed on a column of silica gel. Initial fractions, eluted by hexane, gave ishwarane, $C_{15}H_{24}$, b.p. 80–82°/1 mm, $[\alpha]_D$ – 40·33°. Later fractions, shown to be a mixture when monitored on TLC, on rechromatography over $AgNO_3$-impregnated silica gave aristolochene, $C_{15}H_{24}$ (M^+204), b.p. 85°/0·5 mm, d^{25}0·9477, $n_D{}^{25}$ 1·504, $[\alpha]_D^{25}$ – 76·47°.

The resistance of ishwarane to hydrogenation coupled with the absence of unsaturation in IR and NMR spectra showed that it was tetracyclic. The NMR spectrum of ishwarane exhibited a multiplet at 0·51 for cyclopropane $\underline{H}$, a doublet at 0·73 (3H, J = 6 c/s) for $-CH-\underline{CH}_3$, a singlet at 0·78 (3H) for $>\!\underset{|}{C}-CH_3$ and a singlet at 1·12 (3H) for $>\!C-\underline{CH}_3$ on a cyclopropane ring[4]. The identity of ishwarane in all respects (IR, VPC, rotation and NMR) with a sample of the hydrocarbon, $C_{15}H_{24}$, obtained from ishwarone (I) by a Wolff-Kischner reduction, permitted us to assign unambiguously structure II for ishwarane.

The IR spectrum of aristolochene, different from that of valencene[5] (IV), showed absorptions at 3080, 1648 and 886 cm^{-1} characteristic for a terminal methylene group

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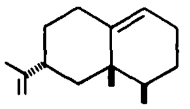
and was lacking in the twin bands around 1380 cm^{-1} due to *gem*-dimethyl group, suggesting the presence of an isopropenyl grouping. A band at 810 cm^{-1} indicated the presence of a trisubstituted double bond. The formation of eudalene on Se-dehydrogenation of aristolochene showed the presence of either a selinane or erermophilane carbon framework. The UV spectrum of aristolochene exhibited only an end absorption and the NMR spectrum showed a doublet (3H, $J = 6$ c/s) at 0·83 ($-\underset{|}{C}H-C\underline{H}_3$), a singlet (3H, $>C-C\underline{H}_3$) at 0·95, a broad signal (3H, olefinic Me) at 1·70 and a broad singlet (2H, terminal methylene) at 4·67. Besides, the NMR spectrum revealed the presence of a vinyl H as a broad multiplet at 5·25 due to the presence of a trisubstituted double bond. The width (8 c/s) at half height of this signal at 5·25 favoured the eremophilane type skeleton for aristolochene.

Selective hydrogenation of aristolochene using deactivated Raney Ni gave a dihydro derivative, $C_{15}H_{26}$, whose NMR spectrum showed the disappearance of the broad olefinic Me signal at 1·70 and the broad singlet at 4·67 due to the terminal methylene protons. However, the multiplet at 5·23 indicated that the vinyl H of the trisubstituted double bond was unaffected. Hydroboration[6] of dihydroaristolochene, followed by oxidation with alkaline H_2O_2 gave a mixture of epimeric alcohols which on further oxidation with CrO_3[7] and homogenization over basic alumina furnished the *trans*-1-decalone V as a liquid, $C_{15}H_{26}O$, b.p. 70°/0·005 mm; $[\alpha]_D - 7{\cdot}24°$, $\nu_{max}CH_2Cl_2$ 1697 (saturated 6-membered ketone) and 1420 cm^{-1} ($-CO\ CH_2$).

The decalone V readily formed a liquid ethylene dithioketal, $C_{17}H_{30}S_2$, which in the mass spectrum showed an intense peak at *m/e* 173 due to the ion[8] VI besides the molecular ion peak at *m/e* 298. In consonance with structure V, the decalone exhibited a positive cotton effect, (*a* + 26). An enantiomer of V had been prepared from eremophilone, but a sample was not available to us for direct comparison.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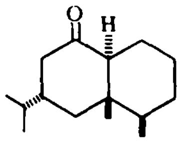
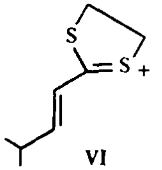
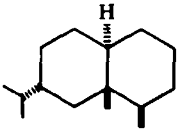

V

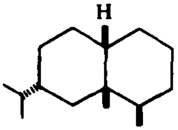

VI

A direct chemical proof for assignment of structure III to aristolochene was obtained in the following manner. Catalytic hydrogenation of aristolochene over PtO_2 in alcohol gave a mixture of stereoisomeric saturated hydrocarbons which showed two peaks in VPC, and these were separated by preparative VPC. The fraction which showed a peak at retention time 68·5 min yielded a colourless liquid $C_{15}H_{26}$, b.p. 100°/0·8 mm, $[\alpha]_D + 10{\cdot}43°$, identical in all respects (IR, VPC and rotation) with an authentic sample of (+) nootkatane (VII) prepared[3] from valencene (IV). The fraction which showed a peak at retention time 72 min was the *cis*-isomer (VIII) obtained as a colourless liquid, $C_{15}H_{26}$, b.p. 98°/0·8 mm $[\alpha]_D + 37{\cdot}09°$.

Thus ishwarane (II) is the first tetracyclic sesquiterpene hydrocarbon based on the eremophilane skeleton and aristolochene (III) represents structurally the simplest member of the biogenetically interesting and steadily growing group of eremophilane type sequiterpenes.[10]



VII



VIII

EXPERIMENTAL

General experimental procedure. M.ps and b.ps are uncorrected. IR spectra were determined on a Perkin–Elmer Model 421 spectrophotometer. NMR measurements were made for CCl_4 solns on a Varian A-60 spectrometer with TMS on an internal standard and the chemical shifts expressed in δ values, VPC was run on a 50′ × ⅛″ (OD) aluminium column using a Varian Aerograph Model 712 fitted with a flame ionization detector. The stationary phase was SE-30 (15%) with a column support of chromosorb W (60–80 mesh). A quantity of 3 ul of the liquid was injected each time and dry N_2 was used as the carrier gas. TLC was performed on Merck silica gel G or silica gel G impregnated with $AgNO_3$ (15%). For visualization, the developed TL chromatograms were sprayed with a 1% soln of vanillin in H_2SO_4 aq (1:1) and heated to 110° for 5 min.

Isolation of ishwarane (II) *and aristolochene* (III) *from* Aristolochia indica. The dark brown oil (214g) obtained by extraction of the roots of *Aristolochia indica* (13·5 kg) with hexane was distilled *in vacuo* and the fractions collected below 110°/mm were found to contain essentially two hydrocarbons, (II and III) besides a small amount of an unknown hydrocarbon. These fractions showed an intense band in the IR at 1648 cm^{-1} (unsaturated) besides a weak CO band at 1707 $cm^{-}1$ due to the presence of I. The hydrocarbon mixture (34g) dissolved in minimum amount of hexane was adsorbed on a column packed with fine Merck silica gel (500g) and eluted slowly with hexane. 15ml fractions were collected and the separation of the mixture of hydrocarbons was monitored on TLC using $AgNO_3$-impregnated silica. Fractions 7–15 gave on distillation ishwarane as a colourless liquid (12·8 g), b.p. 80–82°/1 mm, $[\alpha]_D - 40{\cdot}33$ ($c = 4{\cdot}24$ in $CHCl_3$), identical in all respects with a sample of the hydrocarbon, b.p. 80°/1mm, $[\alpha]_D - 42{\cdot}90°$ ($c = 3{\cdot}72$ in $CHCl_3$) prepared by Wolff-Kishner reduction of ishwarone. UV: λ 211 mμ (ε 210); NMR: 0·51 (m, cyclopropane $\underline{\mathrm{H}}$), 0·73 (d, $J = 6$ c/s, $\text{—}\underset{|}{\mathrm{C}}\mathrm{H}\text{—}\underline{\mathrm{CH}}_3$), 0·78 ($\mathrm{S}\text{—}\overset{|}{\underset{|}{\mathrm{C}}}\text{—}\underline{\mathrm{CH}}_3$) and 1·12 (S, $\text{—}\!\!>\!\mathrm{C}\text{—}\underline{\mathrm{CH}}_3$). (Found: C, 88·26; H, 12·12 $C_{15}H_{24}$ requires: C, 88·16; H, 11·84%). Fractions 16–29 gave a mixture (12·72 g) containing ishwarane and other hydrocarbons. Fractions 30–44 gave an oil (5·5 g) which showed mainly two spots on TLC ($AgNO_3$-silica) and found to be different from ishwarane and this fraction was processed subsequently for the isolation of aristolochene. Later fractions eluted with hexane gave negligible residues. The oil (5·5 g) from fractions 30–44, was chromatographed over $AgNO_3$-silica (110 g) and the column was eluted with hexane. 5 ml fractions were collected and the separation was monitored on TLC ($AgNO_3$-silica) and by NMR. Fractions 1–3 gave a colourless liquid (0·24 g) containing essentially aristolochene and a small quantity of an impurity. Fractions 4–19 gave on distillation pure aristolochene (III) (0·81 g), $C_{15}H_{24}$ (M^+204), b.p. 85°/0·5 mm $d = 0{\cdot}9477$, n_D 1·504, $[\alpha]_D - 76{\cdot}47°$ (neat), R_f ($AgNO_3$-silica), 0·59, dark purple spot, retention time on VPC, 70·5 min with a flow rate of N_2, 97 ml/min and column temp of 250° and press of 35 psi. (Found: C, 88·43; H, 11·98. $C_{15}H_{24}$ requires: C, 88·16; H, 11·84%). Fractions 20–43 gave an oil (0·6 g) which was found to be a mixture of aristolochene and an unidentified hydrocarbon.

Wolff-Kishner reduction of ishwarone (I). To a mixture of ishwarone (5 g) in freshly distilled diethylene glycol (80 ml) Na (3 g) was added in small pieces followed by hydrazine hydrate (10 ml, 85%). After heating under reflux for 2 hr, the reaction mixture was heated to 190–210° for 6 hr. It was then diluted with water and extracted with ether. After removal of the solvent, the residue was dried and subjected to the above sequence once again and the product (3·5 g) was distilled, b.p. 80°/1 mm, $[\alpha]_D - 42{\cdot}90°$ ($c = 3{\cdot}72$ in $CHCl_3$). Found: C, 88·41; H, 11·94. $C_{15}H_{24}$ requires: C, 88·16; H, 11·84%).

Dihydroaristolochene. A soln of aristolochene (0·21 g) in EtOH (10 ml) was shaken in the presence of H_2 at room temp and press over deactivated Raney Ni (0·1 g) for 3 hr until 1 molar equiv of H_2 was absorbed. After removal of the catalyst, the solvent was evaporated *in vacuo* and the residue distilled to yield dihydroaristolochene, b.p. 70–75°/0·2 mm.

Dehydrogenation of aristolochene. An intimate mixture of aristolochene (2 g) and powdered Se (2 g) was heated in a sealed tube at 300–325° for 11 hr and extracted thoroughly with ether. The dark gummy material obtained on evaporation of the solvent was passed through a column of neutral alumina mixed intimately with dry Ag_2O (20 g, 1:1).

The earlier fractions eluted with hexane gave a TNB adduct, m.p. 110°–112°, undepressed on admixture with an authentic sample of eudalene–TNB adduct. The picrate had m.p. 89–91° (lit. m.p. 92°).

Conversion of dihydroaristolochene to the trans *1-decalone* V. The apparatus described by Brown and Zweifel[6] for the external generation of diborane was employed. BF_3-etherate (15 ml) in dry diglyme (10 ml) was added dropwise to a stirred soln of $NaBH_4$ (1·14 g) in diglyme (20 ml). The diborane generated was swept by a slow stream of N_2 through a soln of dihydroaristolochene (350 mg) in dry THF (5 ml) at 0: The reaction mixture was allowed to stand in the ice-chest overnight. The THF soln was treated with water (5 ml) 3N NaOH (8 ml) and 30% H_2O_2 8 ml), with shaking and ice cooling and left at room temp for 2 hr. After addition of more water, the turbid soln was extracted with $CHCl_3$ and the product was a mixture of epimeric alcohols (210 mg). TLC of the mixture showed essentially two spots of nearly the same intensity and hence was chromatographed over silica (12 g). The earlier fractions eluted with benzene gave a small amount of a crystalline compound (56 mg) which was found to be homogeneous on TLC. An analytical sample was made by sublimation at 95°/0·005 mm, m.p. 69–72°, $[\alpha]_D$ + 9·34° (c = 2·2 in $CHCl_3$). (Found: 80·39; H, 12·64. $C_{15}H_{28}O$ requires: C, 80·29; H, 12·58%). Later fractions eluted by benzene gave only a mixture (132 mg) of epimeric alcohols; IR, $\nu_{max}CH_2Cl_2$ 3600 cm^{-1} (OH). This was treated in the cold with a soln of CrO_3 (135 mg) in H_2O (3 drops) and AcOH (2 ml).

After leaving overnight at room temp, excess of CrO_3 was destroyed with $NaHSO_3$ aq and then extracted several times with $CHCl_3$. Evaporation of the combined extracts gave a viscous, colourless liquid which was homogenized by passing a hexane soln through a column of basic alumina. The pure decalone V distilled as a colourless liquid, b.p. 70°/0·005 mm $[\alpha]_D$ − 7·24° (c = 2·21 in $CHCl_3$); IR $\nu_{max}CH_2Cl_2$ 1697 (sat. 6-membered ketone) and 1420 cm^{-1} (CO—CH_2); ORD, pos. cotton effect, a + 26 (c = 0·076 in MeOH).

The semicarbazone, made by treatment with semicarbazide HCl in pyridine at 65°, formed white needles from MeOH, m.p. 195–199°. On recrystallization twice from the same solvent, it had m.p. 199–201° (lit.[9] m.p. 208–210° for the enantiomer). (Found: C, 69·15; H, 10·48. $C_{16}H_{29}ON_3$ requires: C, 68·77; H, 10·46%).

The 2,4-dinitrophenylhydrazone, prepared in the usual manner, on crystallization from MeOH formed yellow needles, m.p. 111–113°. (Found: C, 62·91; H, 7·59. $C_{21}H_{30}O_4N_4$ requires: C, 62·66; H, 7·51%).

The ethylene dithioketal, prepared by treating a soln of V (60 mg) in glacial AcOH, (1 ml) at 0° with a soln of ethanedithiol (200 mg) in AcOH (1 ml) containing BF_3-etherate (0·5 ml), distilled as a colourless liquid, (45 mg), at 95–103°/0·005 mm (bath temp), $[\alpha]_D$ + 18·82° (c = 1·86 in $CHCl_3$). (Found: C, 68·65; H, 10·14. $C_{17}H_{30}S_2$ requires: C, 68·42; H, 10·13%).

(+) *Nootkatane* (VII) *from aristolochene.* A soln of aristolochene (0·535 g) in EtOH (20 ml) was hydrogenated over PtO_2 (50 mg) at 40 psi for 22 hr. After removal of the catalyst, the solvent was evaporated *in vacuo* and the residue distilled at 80°/1 mm to give a mixture of VII and VIII. The fraction which showed a peak at ret. time 68·5 min yielded a colourless liquid, $C_{15}H_{26}$, b.p. 100°/0·8 mm, $[\alpha]_D$ + 10·43° (c = 2·26 in $CHCl_3$) identical in all respects with an authentic specimen of VII, $[\alpha]_D$ + 10·07° (c = 2·025) prepared[3] from valencene. (Found: C, 86·27; H, 13·58. $C_{15}H_{28}$ requires: C, 86·46; H, 13·54%).

The fraction which showed a peak at ret. time 72 min was the *cis*-isomer VIII; distilled as a colourless liquid, $C_{15}H_{26}$, b.p. 98°/0·8 mm, $[\alpha]_D$ + 37·09° (c = 2·20 in $CHCl_3$). (Found: C, 86·80; H, 13·50. $C_{15}H_{28}$ requires: C, 86·46; H, 13·54%).

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