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STUDIES IN THE STEROIDAL CONSTITUENTS OF THE SEEDS OF *ABRUS PRECATORIUS* LINN. (SCARLET VARIETY)

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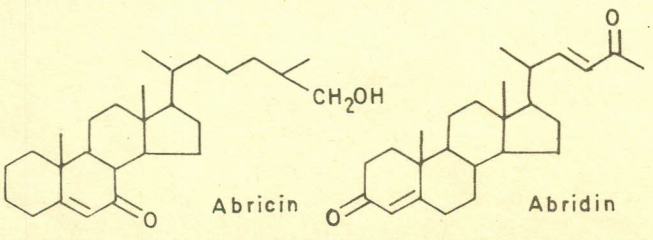
Exhaustive chromatography of the unsaponifiable fraction of the fatty constituents of Abrus seeds have led to the isolation of two new steroids named as abricin and abridin. Cycloartenol, β-amyrin, campesterol, cholesterol, stigmasterol, β-sitosterol and an oily hydrocarbon β-squalene have also been isolated and identified, which were spotted earlier.

Taking into account the medicinal importance attached to the seeds and various parts of Abrus in the indigenous system of medicine for the treatment of a variety of ailments, and in view particularly of the long active contraceptive property claimed for the seeds in folklore, an exhaustive study of the chemical constituents of the scarlet variety of seeds has been undertaken. Earlier workers in the field, have mainly devoted their attention to its proteinous toxic principle, abrin [1], and the weakly basic component abrine (Ghatak and Kaul) [2], the structure of which was established as N-methyl tryptophane by Hoshino *et al.* [3]. Later on, a considerable amount of chemical work was carried out by other workers to study the alkaloids [2, 4], amino acids [5, 6, 7,], carbohydrates [7, 8, 9], fatty acids [10] and steroids [11]. More recently Rupwala *et al.* [12] have referred to the antifertility activity of the total oil of Abrus seeds.

As a result of the the present work ultimately based on procedures described in the Experimental, eight crystalline steroidal components have been isolated. Two of them appear to be new and have been provisionally named as abricin and abridin, whereas the others were identified as β-sitosterol, stigmasterol, cholesterol, cycloartenol and β-amyrin, the presence of which was previously indicated by Lefar *et al.* through GLC [11]. The following structures of the new compounds have been arrived at through spectral studies and chemical reactions.

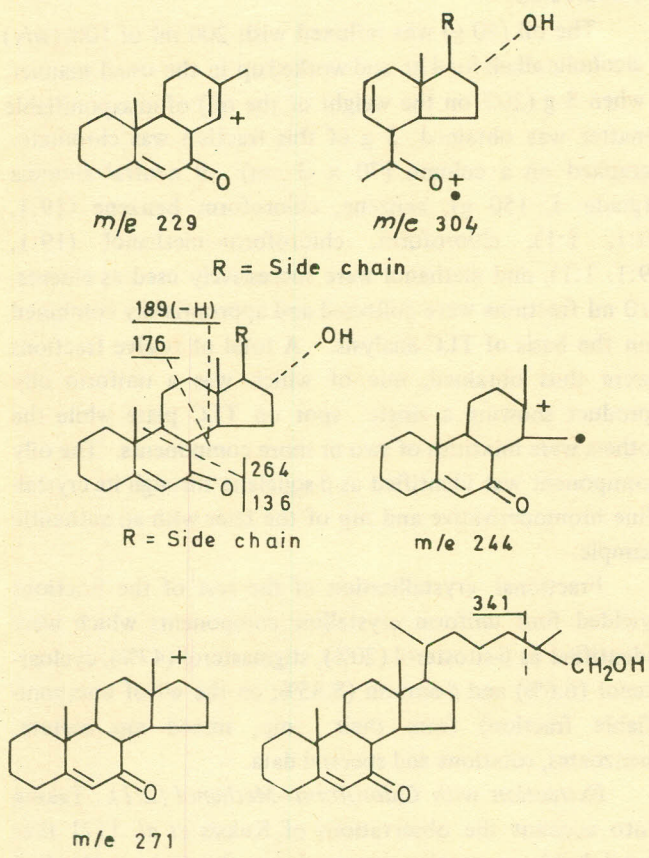


Characterisation of Abricin. Abricin was obtained from a powdery deposit (mp 166–70°) that settled down on keeping an alcoholic solution of the unsaponifiable matter at room temperature. On dissolving it in a mixture of 1:9 glacial acetic acid – ethanol and cooling the solution after concentration, when it came out as a shining white crystallizate. It would thus appear that abricin does not occur as such in the seed and is a degradation product of the amorphous powder referred to above, which failed to yield any uniform crystalline constituent. In contrast to the amorphous powder which is insoluble in all bench solvents, abricin is readily soluble in ether, petroleum ether and alcohol, and after repeated crystallizations from ethanol it melted at 85° and analysed for $C_{27}H_{44}O_2$. It gave positive tests for sterol, formed an acetate (mp 105–6°) and a 2:4-dinitrophenyl hydrazone (mp 95–6°) indicating the presence of an alcoholic and a ketonic function respectively. Jones oxidation of abricin aforded the corresponding acid, mp 128°, demonstrating the primary nature of the hydroxyl group. It absorbed one mole of bromine showing the presence of an ethylenic bond in the molecule.

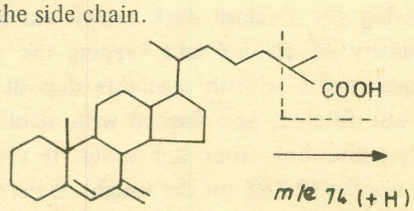
The IR spectrum of abricin showed peaks for –OH group (3400 cm^{-1}), conjugated carbonyl (1680 cm^{-1}), and C=C (1580 cm^{-1}) apart from other absorptions at 2980, 3010, 870 and 1410–1470 cm^{-1}. The UV spectrum was also indicative of conjugated carbonyl chromophore in the six membered ring with λ_{max} at 238 nm. Considering the fact that Δ^5-7-one shows maximum at 239 nm [13], an α-hydroxy Δ^5-7-one structure was proposed as a working hypothesis.

The mass spectrum showed fragments at *m/e* 400=M^+, 385 = M^+–CH_3, 382 = M^+–H_2O, 367 = M^+–(CH_3 + H_2O). A fragment at *m/e* 229 was indicative of residue of rings A, B and C with a carbonyl group in ring A or B in accord with the observations of Elliot [14] while absence of ions at *m/e* 122, 109, 358 (M-42), 124, 105, 135 and 149 excluded to a large extent the possibility of Δ^2-1, Δ^1-3,

Δ^4-3, Δ^5-4 and Δ^4-6 ketones. The alkenic function and the carbonyl group could be located at Δ^5-C_7 on the evidence of relative intensities of the ions at *m/e* 304, 176, 189, 264 and 136 (M^+-264) respectively. Ions at *m/e* 229, 244 and 271 further indicated that the hydroxyl group is not located in the nucleus. Information regarding the presence of $-CH_2OH$ group was obtained from a fragement at *m/e* 370 (M^+-30) which left C-21 and C-26 as the only possible loci for the hydroxyl group. A fragment at *m/e* 341 (M^+-59) was also observed, possibly resulting from the side-chain. The structural position in respect of its various possible fragments is shown below:



Evidence in favour of the hydroxyl group at C_{26} was furnished by the mass spectrum of the acid resulting from the oxidation of abricin, since the McLafferty rearrangement resulted in an *m/e* 74 fragment [15]. The molecular ion was observed at *m/e* 414, apart from other fragments corresponding to those of abricin with an increment of 14 amu, in the side chain.



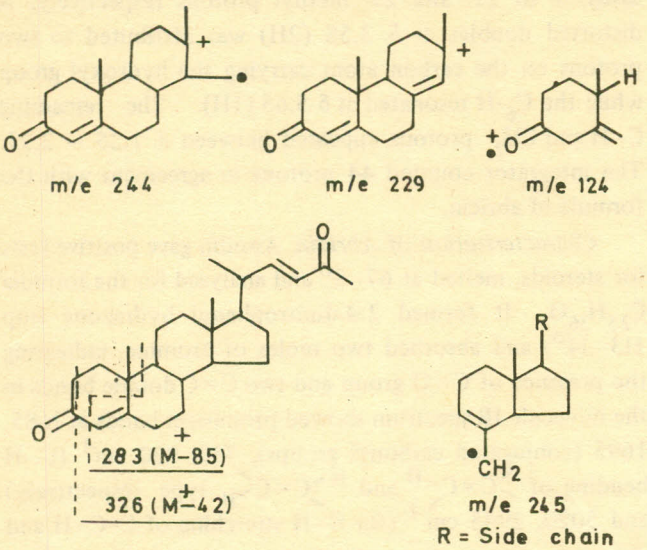
The H^1-NMR provided almost conclusive evidence in favour of this structure. Four signals were observed for methyl groups, two singlets at δ 0.71 (3H), and δ 1.12 (3H) were assigned to 18-and 19- angular methyl groups while two peaks at δ 0.86 (3H) and δ 0.95 (3H) have been assigned to 21- and 27- methyl protons respectively. A distorted doublet at δ 3.58 (2H) was attributed to two protons on the carbon atom carrying the hydroxyl group while the C_6-H resonated at δ 5.65 (1H). The remaining C–H and CH_2 protons appeared between δ 1.25 – 2.23. The integrator counted 44 protons in agreement with the formula of abricin.

Characterization of Abridin. Abridin gave positive tests for steroids, melted at 67–8° and analysed for the formula $C_{25}H_{36}O_2$. It formed 2:4-dinitrophenyl hydrazone (mp 113–14°) and absorbed two moles of bromine, indicating the presence of C=O group and two C=C double bonds in the molecule. IR spectrum showed prominent bands at 1685, 1695 (conjugated carbonyl groups), 730, 930 cm^{-1} (C–H bending of $\rangle C=C\langle^{H}$ and $^{H}\rangle C=C\langle_{H}$ type respectively) and 3020, 2945 cm^{-1} (for C–H stretching of C=C–H and C–C–H type respectively). Peaks for –COOH, –COOR and –OH groups were not observed and, therefore, two carbonyl functions were suggested in the molecule. In the UV spectrum λ_{max} was observed at 241 nm showing a close resemblance with cholest - Δ^4 - 3 - one (λ_{max} 241 nm) [13]. One more band appeared between 226–227 nm.

A good deal of information, concerning the position of functional groups and double bonds was afforded from the prominent fragments of the mass spectrum. Peaks were observed at *m/e* 368=M^+, 353= M^+-CH_3 and *m/e* 271 (M^+-side chain) which suggested a side chain of C_6H_9O (97 amu) which was also supported by a fragment at *m/e* 97. The peak at *m/e* 269 = M^+-(side-chain + 2H) indicated the presence of one double bond in the side-chain while the fragment at *m/e* 298 (M^+–70) due to the cleavage of side chain at C-20, C-22 with hydrogen migration to produce a loss of C_4H_6O (*m/e* 70) was indicative of a $\Delta^{22\text{-}23}$ unsaturation. Placement of one double bond at C_{22}–C_{23} in the side-chain suggested C_{24} as the possible position for oxygen. Furthermore, peaks at *m/e* 43 (terminal $C^{=O}$–CH_3) and at *m/e* 325 (M^+-43) also supported 24-one structure, suggesting a $\Delta^{22(23)}$-24-one structure in the side-chain, which was supported by the second band of the UV spectrum between 226–227 nm (calculated 227 nm for $\Delta^{22(23)}$-24-one).

The fragment of *m/e* 244 suggested that the second carbonyl group was not in the ring D, while the ion at *m/e* 229 was again attributed to the residuum of rings A, B and C with the carbonyl group in ring A or B (loc cit). The fragmentation of abridin afforded prominent ions at *m/e* 124, 326 (M^+-42), 283 (M^+-85) and *m/e* 245 (148 +

side-chain), which were in complete agreement with the Δ^4-3-one structure in accord with the observations of Djerassi *et al.* [16] (e.g. methyl 3-keto- Δ^4 - cholanate). The various possible fragments are shown below:



Supporting evidence was afforded from the H^1-NMR spectrum, that showed two singlet-s at δ 0.71 (3H) and δ 1.13 (3H) for 18- and 19- angular methyl protons respectively. A peak at δ 0.93 (3H) was assigned to the 21- methyl protons while a singlet at δ 2.2 was assigned to the 25-methyl protons in agreement with $-C^{O}- CH_3$ tructure in the molecule. A shift at δ 5.71 (1H) and a doublet at δ 6.61 (1H) was assigned to C_4- and C_{23}- protons respectively, while the C_{22}- proton resonated at δ 7.03 (1H).

Physiological studies in the antifertility action of abridin by Dr. M.E.Hamdard Miss Atiya Zia and Prof. M.H. Qazi have shown that with a dosage of 1.5 mg /kg, 100% sterility occurs in rats (unpublished work). Taking this finding into account and also the roughly corresponding dosage of antifertility activity of the total oil recorded by Rupwala *et al.* (loc cit.) it would appear that the reputed contraceptive activity would require very large quantities of the seeds for an adult woman. It could thus be concluded that the folklore claim is either not tenable, or that the antifertility activity is due to the combined effect of the constituents which also include the toxic principle. The present work, however, may well lead to fruitful structural and synthetic studies and significantly contribute towards population planning measures.

EXPERIMENTAL

TLC was carried out on silica gel (Merck 60 F_{254}) and spots were located either under UV lamp or by iodine vapours. IR spectra (KBr) were recorded on a Unicam SP 200 G spectrophotometer. UV spectra were obtained for solutions in methanol on Unicam SP 800. NMR spectra were recorded for solutions in H^2-chloroform on Jeol PMX 13 (tetramethylsilane as internal reference). Mass spectra were measured on VG Micromass 12. Rotations were measured for solutions in chloroform. Mps were recorded in glass capillary tubes and are uncorrected.

Extraction with Petroleum Ether. The scarlet seeds of *Abrus precatorius* (8 kg, 30 mesh) were exhaustively extracted with petroleum ether (40–60°) at room temperature. Removal of the solvent from the combined extracts finally under reduced pressure gave about 100 g of a yellow coloured oil.

The oil (40 g) was refluxed with 200 ml of 10% (w/v) alcoholic alkali for 4 hr and worked up in the usual manner, when 8 g (20% on the weight of the oil) of unsaponifiable matter was obtained. 5 g of this fraction was chromatographed on a column (70 x 3 cm) of neutral alumina (grade I, 150 g); benzene, chloroform–benzene (19:1, 9:1, 1:1), chloroform, chloroform–methanol (19:1, 9:1, 1:1), and methanol were successively used as eluents, 10 ml fractions were collected and appropriately combined on the basis of TLC analysis. A total of twelve fractions were thus obtained, one of which was a uniform oily product showing a single spot on TLC plate while the others were mixtures of two or more components. The oily component was identified as β-squalene through its crystalline bromoderivative and mp of the later with an authentic sample.

Fractional crystallisation of the rest of the fractions yielded four uniform crystalline components which were identified as β-sitosterol (20%), stigmasterol (47%), cycloartenol (6.6%) and β-amyrin (8.85%; on the wt of unsaponifiable fraction) from their mp, mixed mp acetate, benzoates, rotations and spectral data.

Extraction with Chloroform–Methanol (2:1). Taking into account the observations of Kuksis *et al.* [17] that total lipids cannot be extracted completely by individual lipid solvents, 20 kg powdered seeds (30 mesh) were repeatedly percolated in another working with a mixture of methanol–chloroform in 1:2 ratio. On removal of the solvent from the combined percolates under reduced pressure about 700 g (3.5%) of thickish liquid was obtained.

On dissolving the thickish dark brown extract in a minimum quantity of alcohol and keeping the solution at room temperature a whitish insoluble deposit settled down which was filtered, and washed with alcohol. On repeated recrystallisations from hot water, it ultimately formed white needles (0.35% on the weight of the extract) melting at 295° (decomp) corresponding to the melting point of abrine reported earlier (loc cit). The residue from

the filtrate and washings was completely soluble in petroleum ether. On its saponification with 6% alcoholic alkali on boiling water-bath for 3 hr, and working up the hydrolysate in the usual manner, 20% of an unsaponifiable fraction was obtained which when taken in alcohol and kept at room temperature gave an amorphous dirty white powder that failed to crystallise and melted at 166-70°. It was insoluble in all the bench solvents and water, but formed a clear solution in acetic acid–ethanol (1:9) and on concentration deposited shining white plates of abricin melting at 85°.

The main bulk of the unsaponifiable fraction after separation of the above compound, was subjected to chromatographic techniques of isolation and 5 g of the unsaponifiable fraction was chromatographed on column (71 x 3 cm) of silica gel (200 g; Merck), [eluent: petroleum ether, petroleum ether–ether (49:1, 19:1, 9:1, 3:1, 1:1), ether, ether–methanol (1:1), and methanol]. Fractions (10 ml) were analysed by TLC and combined accordingly when six single-spot components were obtained, while the rest were mixtures of more than two components. These were combined, and rechromatographed on silica gel (Merck) and silica gel impregnated with silver nitrate, furnishing a total of eleven single components Out of these, four compounds could be crystallised while the rest of them retained an oily character. One of these oily components could be characterised as β-squalene on the basis of mp and mixed mp of the bromoderivative with that of an authentic sample. One of the four crystalline components appeared to be new and has been provisionally named as abridin, as stated in the Introduction, while the remaining three were characterised as cholesterol (1.45%), stigmasterol (47%) and β-sitosterol (20%; of the unsaponifiable fraction) on comparing their physical and chemical data with those of authentic samples.

In another scheme the unsaponifiable fraction (4 g) was chromatographed on a column (60 x 2.5 cm) of magnessium silicate using ether–petroleum ether (1:9,1:1), ether, and methanol. Following this procedure campesterol could also be isolated in addition to other components referred to in the earlier working.

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