UDC 633.88 (540)

STUDIES ON THE MEDICINAL PLANTS OF INDIA*

M. L. Dhar

Khimiya Prirodnykh Soedinenii, Vol. 5, No. 5, pp. 412-427, 1969

Diverse geographic conditions in India favor the vigorous growth of almost all forms of plant life. Consequently, it is not surprising that the ancient medicinal preparations of India were obtained mainly from plants. More than 2000 years ago Susruta and Sharaka drew up exhaustive treatises in which plant materials used as medicines were described in detail. In India there are more than 2200 species of plants to which medicinal properties have been ascribed.

The first attempts to analyze an ancient drug were made in India in the 1920's by a group of scientists in the Institute of Tropical Medicine at Calcutta led by Prof. R. N. Chopra and his colleagues, by Dr. Sudhamoy Gosh, by Dr. B. Mukherjee, and others. This group investigated the medicinal plants of India from all aspects. At this time, microbiological and pharmacological technique was only beginning its development in the laboratories of India, and the chemical investigation of the plant material was limited mainly to the isolation of crystalline substances which were then subjected to biological tests. Thus, Chopra's school determined the activity of a number of plant products—for example, the activity of the steroid base conessine (from Holarrhena antidysenterica), used for the treatment of amebic dysentery.

In the middle of the thirties, Dr. Siddiqui and his colleagues began a chemical investigation of a number of medicinal plants, but the majority of substances isolated by them were not studied biologically. A leading contribution to science was their work on the alkaloids of Rauwolfia serpentina although the preparation of reserpine—the main hypotensive base of this plant—and the determination of its therapeutic activity was carried out by Schlittler and Bein at the beginning of the fifties.

An enormous advance in the study of medicinal plants during the last thirty years has been the work of Prof. Seshardri on psoralen and khellin, the investigations of Prof. Govindachari and Prof. Vora on jatamansone, those of Prof. A. Chatterjee on the indole alkaloids, of Prof. Rangaswami on cardiac glycosides from Thevethia nariifolia, of Prof. Chakravarti on hormonal steroids, the discoveries of Prof. Gujral of the antiinflammatory activity of the saponins of Glycyrrhiza glabra, and the work of Dr. Bhide on the tranquilizing action of steroids from Paspalum scrobiculatum and of Dr. Dutta and of I. K. Shete on berberine and other natural substances.

In 1951 the Central Drug Research Institute, combining botanists, chemists, microbiologists, and pharmacologists, was organized. In 1963 a laboratory under the direction of Prof. T. R. Govindachari was created in Bombay with functions similar to those of the Central Institute.

For a quarter of a century now, India has had a powerful school on the chemistry of natural substances directed by such leading scientists as Prof. T. R. Seshadri, Prof. K. Venkataraman, Prof. D. K. Banerjee, Prof. S. K. Bhattachari, Prof. Sukh Dev, Prof. P. K. Datta, Prof. R. N. Chakravarti, Prof. A. Chatterjee, and Dr. S. P. Popli. A great contribution to the work of this school was made by the late Prof. Bardan.

We began our work with a study of those plants which were considered to possess some biological activity. Thus, we tested more than 50 medicinal plants.

At the present time to accelerate investigations and the introduction of medicinal preparations, we proceed in the following way: we extract the plant with 50% ethanol, and subject the extracts to biological screening with a system of 66 pharmacological tests to detect antifungal, antiviral, antibacterial, antiprotozoal, antihelminthic, anticancer, contraceptive, and hypoglycemic activities. In the course of the last four and a half years (1964-1968) more than 1100 plants have been collected and about 800 have been subjected to screening; anticancer activity has been confirmed in 34 and other activities in 30.

The subsequent stage, the stage of the isolation of the biologically active components, is now carried out for the most promising plants. The isolation of the active components and the determination of their chemical structures has been achieved for the plants Croton bonspalandianum and Arnebia nobilis. At present, a detailed pharmacological and biological study of these compounds is being carried out.

Let us consider some of the investigations carried out in the Central Drug Institute in the last 17 years on alkaloids, triterpenes, steroids, other polycyclic compounds, nucleic acids, and peptides.

---

* From a Report at the Indo-Soviet Symposium on the Chemistry of Natural Compounds in Tashkent, September, 1968.

N_γ-Methyl-N_α-ethylbrevicarine N_β-methiodide (IIIj). A solution of 0.5 g of IIIh in ethanol was treated with a 10% solution of NaOH. The bright yellow precipitate of the methiodide was recrystallized from ethanol. Mp 180° C (decomp.).

Found, %: C 55.32; H 6.71; N 9.19; I 27.98. Calculated for $C_{21}H_{30}N_3I$, %: C 55.87; H 6.65; N 9.31; I 28.15.

N_γ-Methyl-N_α-ethylbrevicarine N_β,N_γ-dimethiodide (IIIl). A solution of 0.2 g of IIIj in ethanol was heated with CH_3I. Mp 178–179° C (decomp.) (ethanol).

Found, %: N 7.08. Calculated for $C_{22}H_{33}N_3I_2$, %: N 7.08.

The alkyl iodide derivatives of brevicarine with different sequences of alkyl radicals were obtained similarly: brevicarine N_β,N_γ-diethiodide (IIIg), mp 243–245° C (aqueous ethanol); N_β-ethylbrevicarine N_γ-ethiodide (IVd), mp 256–258° C (aqueous ethanol); N_α-methylbrevicarine N_β,N_γ-diethiodide (IIIm), mp 208–210° C (aqueous ethanol); N_γ-ethyl-N_α-methylbrevicarine N_β-ethiodide (IIIk), mp 195–197° C (aqueous ethanol); N_α,N_γ-dimethylbrevicarine N_β,N_γ-diethiodide, mp 170° C (ethanol).

The compositions of all the compounds given were confirmed by elementary analysis.

Conclusions

1. The alkylation reaction of brevicarine have been studied in detail.

The structural formula I proposed for brevicarine [1-methyl-4-(4-methylaminobutyl)-β-carboline] has been confirmed by NMR and mass spectroscopy.

REFERENCES

1. I. V. Terent'eva and A. V. Borovkov, collection: Alkaloid-bearing Plants of Moldavia [in Russian], Kishinev, 41, 1960.

2. M. D. Mashkovskii, Medicinal Plants, supplement 1, [in Russian], Moscow, 88, 1964.

3. J. Spenser, Chem. Soc., 3659–3663, 1956.

4. I. V. Terent'eva and P. A. Vember, collection: Alkaloid-bearing Plants of Moldavia [in Russian], Kishinev, 35, 1960.

5. H. Budzikiewicz, C. Djerassi, and D. Williams, Interpretation of Mass Spectra of Organic Compounds [Russian translation], Moscow, 84, 91, 1966.

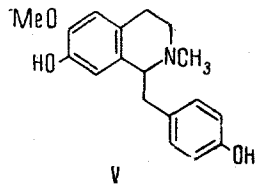
5a. H. Budzikiewicz, C. Djerassi, and D. Williams, Interpretation of Mass Spectra of Organic Compounds [Russian translation], Moscow, 302-307, 1966.

6. P. A. Vember, I. V. Terent'eva, and G. V. Lazur'evskii, KhPS [Chemistry of Natural Compounds], 3, 249, 19 1967.

22 April 1968

Institute of Chemistry AS Moldav SSR

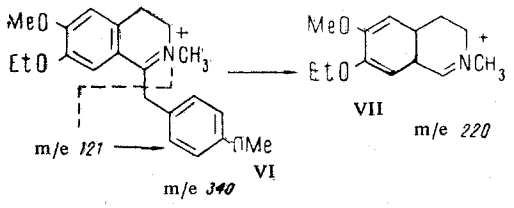
Ethylated hyatinin was treated with sodium in liquid ammonia. A phenolic and a nonphenolic fraction were isolated. The phenolic compound proved to be N-methylcoclaurine (thin-layer chromatogram on silica gel) and the methiodide of its O,O'-dimethyl derivative with mp 131° C was identical with an authentic sample of the methiodide of O,O'-trimethylcoclaurine, i.e., the phenolic fragment possesses the structure of N-methylcoclaurine (V).



V

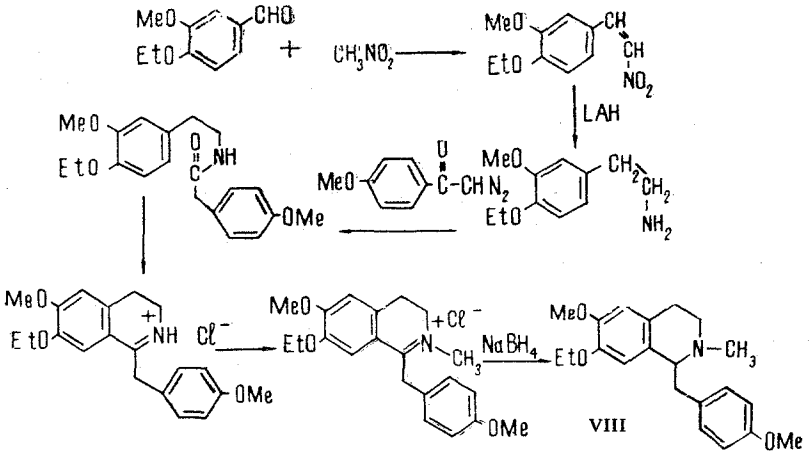
Scheme 3

In the NMR spectrum of the nonphenolic cleavage product there are six protons in the aromatic region, two three-proton singlets with τ 6.12 and 6.18 (2 OMe), a three-proton singlet at 7.43 (N—Me), and a three-proton triplet at 8.67 (J = 7 Hz) (OCH_2—CH_3). The absence of an OCH_3 signal at τ 6.4–6.5 showed that the OC_2H_5 group is present in position 7. This means that the free hydroxyl (phenol) group in hyatinin also occupies position 7. The deuteration of hyatinin shows that there are no protons in the ortho and para positions with respect to the phenolic group. Position 7 for the phenolic hydroxyl group is also confirmed by the mass spectrum of the nonphenolic compound which, apart from a weak molecular peak at 341 (M) and a peak of medium intensity at 340, contains strong peaks of ions with m/e 320, 190, 177, and 121. The (M – 1) peak with m/e 340 corresponds to the ion VI, and the main peak with m/e 220 to the ion VII. The formation of this type of ions is characteristic for the fragmentation of the benzyltetrahydroisoquinolines. The ion with m/e 190 could apparently arise by the further loss of an OCH_3 group together with the migration of a proton from the ion VIII, and the ion with m/e 121 in the decomposition of the ion VI (Scheme 4, broken line).



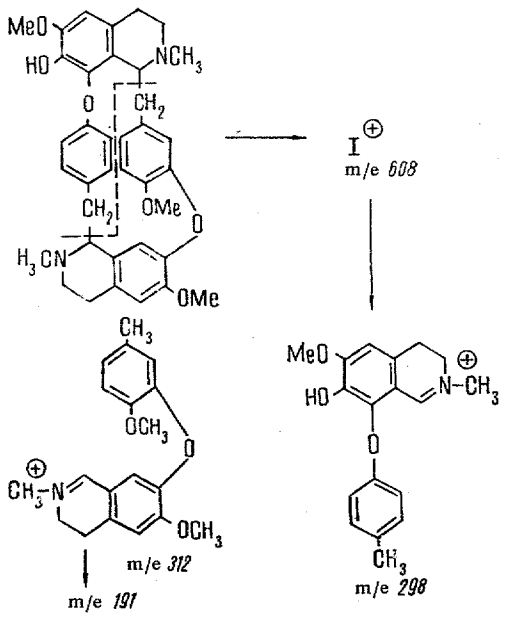
Scheme 4

On the basis of these results, the nonphenolic fragment may be represented by formula VIII. This structure was confirmed by its synthesis (see Scheme 5).



Scheme 5

The determination of the structures of the phenolic and nonphenolic fragments showed the structure of hyatinin as 4"-O-methylberbeerine. This structure is in complete agreement with the NMR spectra of hyatinin and its ethyl ether. This is also shown by the mass spectrum of I with the main ions at m/e 608 (M), 312, 298, 191, 190, 174, 162, 146, and 245. The ions with m/e 608, 312, 298, and 191 are shown in Scheme 6.



Scheme 6

In the mass spectrum of the ethyl ether of hyatinin, the peak (M^+) is shifted to m/e 636, and the main peaks of the two halves appear at m/e 326 and 312, respectively, as was to be expected.

The phenolic and nonphenolic fragments from the reduction of the ethyl ether of hyatinin with sodium in liquid ammonia are optically inactive. Before the finding of this fact, the optical inactivity of hyatinin was assumed to be due to the (+, −) nature of the two corresponding coclaurine fragments, which compensate one another's optical activity. However, the inactivity of the fragments shows that natural hyatinin is a racemic mixture. The structure of hyatin and hyatidine was established by the same route.

From the roots of this plant we have isolated two water-soluble quaternary bases. The main quaternary alkaloid, cissamine chloride, has been characterized as a protoberberine alkaloid and proved to be identical with cyclanoline chloride. The substance was obtained previously by Tomita et al. [8] from Stephania tetrandra. This is the first case of the isolation of a protoberberine alkaloid from Cissampelos pariera.

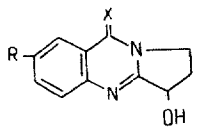
Biological Activity

Among the various Cissampelos alkaloids studied, hyatin methiodide gave the most promising results as a substance causing neuromuscular blockage.

The muscle-weakening effect of hyatin methiodide (HMI) was tested on several species of animals. The pharmacological tests included specific tests on muscles, the drooping head method in rabbits, a preparation of the sciatic nerve in cats and dogs, and a phrenic-nerve preparation in rats.

In a comparison of the biological activities of hyatin methiodide and d-tubocurarine chloride (TC) it was found that although when the former was injected into mice and rabbits it acted more strongly than d-tubocurarine chloride, its activity in man was only half that of d-tubocurarine. Nevertheless, a clinical test of this substance (300 patients) has shown that it is a good neuromuscular blocking agent [9].

Adhatoda vasica Nees. In the Indian medicinal system the expectorant and antispasmodic action on the bronchii of A. vasica was known. Two dihydroquinazoline alkaloids, vasicine and vasicinol, isolated from the roots of A. vasica proved to be identical with peganine and 6-hydroxypeganine IX and X, respectively [10, 11].



Vasicine ≡ peganine IX R=H, X=H_2

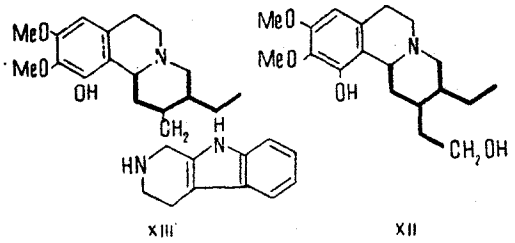
Vasicinol ≡ G hydroxypeganine X R=OH, X=H_2

Vasicinone XI R=H, X=O

Scheme 7

Both these alkaloids and vasicinone (XI) have been investigated for pharmacological activity. Vasicinone and, to a smaller extent, vasicinol show only a weak bronchodilatating activity.

Alangium lamarkii Thw. Three new alkaloids have been isolated from the leaves of this plant: ankorine $C_{19}H_{29}NO_4$ (XII) with mp 174–176° C, alangimarkine $C_{29}H_{37}N_3O_3$ (XIII) with mp 184–186° C, and dihydroprotoemetine $C_{19}H_{29}NO_3$ [12]. Ankorine and alangimarkine have structures XII and XIII, respectively.



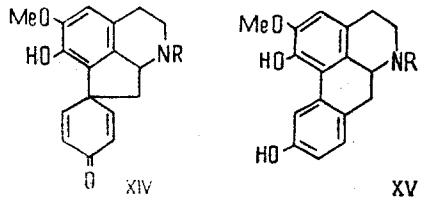
XIII XII

Scheme 8

The C_9 fragment of terpenoid origin (shown by heavy lines in Scheme 8) is the same as in the ipecac alkaloids. The question of the significance of this fragment has been considered by Dr. Popli.

The types of alkaloids present in this plant were first shown by Dr. Pakrashi (Calcutta), who identified the alkaloid sephaline.

Croton sparsiflorus Morong. In an investigation of Cr. spars. (C. bonspalandianum), it was found that the hypotensive activity of an extract of the plant is due to the presence of a new proaporphine base N-methylcrotsparine $C_{18}H_{19}NO_3$ [mp 223–225° C, $[\alpha]_D$ −113° (chloroform)], which can easily be obtained by the N-methylation (formic acid–formaldehyde) of the more common crotsparine $C_{17}H_{17}NO_3$ with mp 193–195° C $[\alpha]_D$ −30° (chloroform). The structure of these two alkaloids as XIV (R = Me and H, respectively) was readily established on the basis of their UV, IR, NMR, and mass spectra and the results of a study of acid rearrangement for the apocrotsparines XV [13].



XIV XV

Crotsparine XIV (R = H); apocrotsparine XV (R = H); N-methylcrotsparine XIV (R = Me); apo-N-methylcrotsparine XV (R = Me).

Scheme 9

It was established pharmacologically that N-methylcrotsparine on intravenous injection into cats in a dose of 5 mg/kg causes a fall in the blood pressure by 50% which lasts 3 hr. This hypotensive effect is not eliminated by sectioning of the spinal cord, tight bandaging, vagotomy, or even by the administration of atropine, antisan, and N-methylcrotsparine, and it partially blocks the reaction to epinephrine and norepinephrine. The corresponding N-methyldihydrocrotsparine, also isolated in small amounts, causes a sharper fall in the blood pressure.

Triterpenes

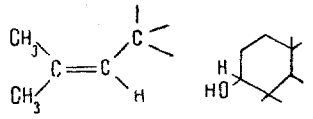
Bacopa monnieri Wettst (family Scrophulariaceae) has long been celebrated in India as an agent for the treatment of psychiatric diseases and epilepsy. However, as has been established in our Institute, the extracts and glycosides of this plant do not possess an appreciable tranquilizing action. Furthermore, the nonsaponin water-soluble fraction possesses anticancer activity (Walker's carcinosarcoma in the rat).

Careful chromatography of the saponin fraction on silica gel gave two saponins, which have been called bacoside A and bacoside B [14]. The two saponins have the same empirical formula and on acid hydrolysis give one and the same triterpenoid aglycone, which has been called bacogenin. These substances proved to be arabinosyl-glycosyl derivatives differing only in the configuration of the carbohydrate residue [15].

Preliminary investigations [15] of bacogenin A have shown that it contains three hydroxyl groups, one of which does not acetylate, while none of them is adjacent to another. A ketone group is present in a five-membered ring and

its function is hindered, since the reduction of bacogenin A with lithium aluminum hydride leads to an axial hydroxyl resistant to acetylation. On the basis of the empirical formula $C_{30}H_{50}O_4$, the presence of a double bond, and a ketone function, and a $C_{(8)}$ CH_3 group (NMR spectrum) it has been deduced that bacogenin A is a tetracyclic triterpene.

Further information on the structure of this compound was obtained by a detailed study of the NMR spectra of bacogenin A_1 and its acetate. In the spectrum there is a multiplet of an olefinic proton at τ 4.67 (320Hz) split by a C—CH_3 group (τ 8.4; 96 Hz, and 8.49; 91 Hz) since the splitting disappears with double resonance. All this shows the presence of the structural unit XVI, which was confirmed by the formation of acetone on oxidation with lead tetraacetate of the diol produced by the treatment of bacogenin A_1 with osmium tetroxide.



XVI XVII

Scheme 10

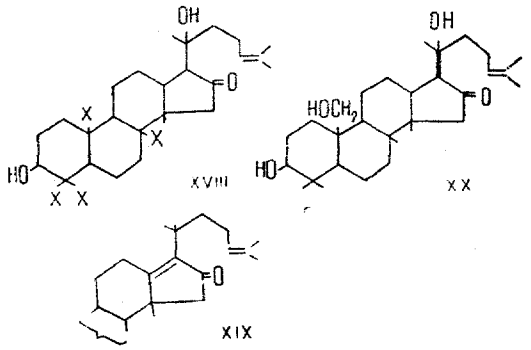
A broad signal at τ 6.8 (192 Hz), due to a single proton on a carbon atom with an oxygen function, shifts on acetylation in the weak-field direction by 60 Hz. Consequently the hydroxyl group occupies position 3, as is generally the case in the triterpenes (XVII).

A quartet with a center at approximately τ 6 (240 Hz) in the spectrum of acetylated bacogenin A_1 shifts in the weak field direction by 20 Hz, showing the presence in the molecule of a hydroxymethylene function.

In addition to signals at τ 8.49 (91 Hz) and τ 8.4 (96 Hz), relating to an isopropylidene group, there is a signal of a methyl group with unusually high resonance at τ 8.62 (83 Hz) which suggests 1,3-interaction of the oxygen function with one methyl group. For this methyl group must be located at $C_{(14)}$ and the carbonyl function in position 16, which makes bacogenin A_1 one of the dammarene series of triterpenes.

The methyl signal at τ 8.74 (76 Hz) may belong to a methyl group located on a carbon atom having an oxygen function as in the case of the 20-hydroxydammarenes (XVIII), which is confirmed by the formation of an enone (XIX) on dehydration.

The shift of the signal of the methyl group on acetylation (Lehn's data) and the fact that bacogenin A cannot give an acetonide shows the C_{10} position for the hydroxymethyl function. Consequently, bacogenin A_1 is 3,10,20-trihydroxy-16-oxodammar-24-ene (XX).

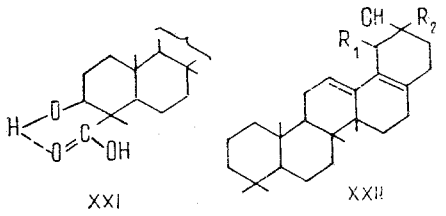


XVIII XX XIX

Scheme 11

Randia dumetorum Lam. (family Rubiaceae) [16]. The acid hydrolysis of the mixture of saponins isolated from the bark of this plant gave a mixture of acid sapogenins. Thin-layer chromatography on silica gel plates showed the presence of two main components. The slow-moving component has been called randialic acid A and the faster-moving component randialic acid B. Randialic acid B was obtained by the fractional crystallization of a mixture of sapogenins and the methyl esters of both acids by the fractional crystallization of a methylated mixture of the sapogenins.

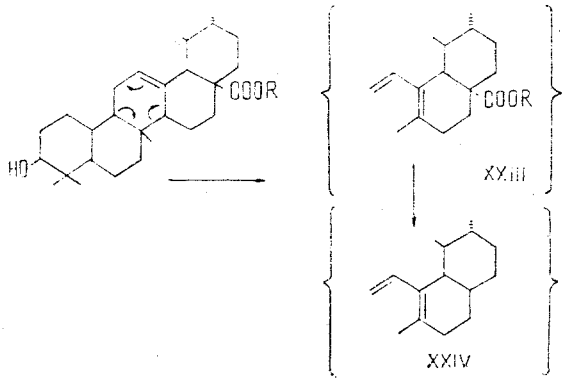
Randialic acid B and its derivatives are strongly dextrorotatory compounds and have an unusual maximum of relatively low intensity (at about 228 mμ) in the UV spectrum. It was assumed that the high dextrorotation could arise as a consequence of the closeness of the conjugated system to the optical center or through a hydrogen bond between the carboxyl and hydroxyl functions, as in the boswellic acids (XXI). In our case, the oxoester obtained by the oxidation of randialic acid B with Jones's reagent should be readily decarboxylated on conversion into the oxoacid, giving a ketone as the final product.



Scheme 12

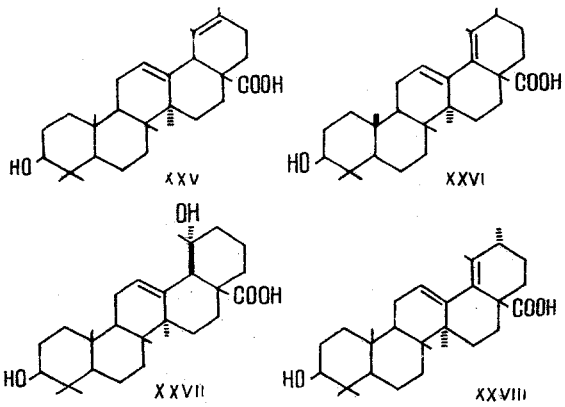
In actual fact, decarboxylation took place readily, but the product had UV maxima at 237, 244, and 253 mμ (log ε 18,500, 19,500, and 12,500, respectively). This is characteristic for the spectra of the heteroangular dienes of type XXII obtained from ursane and oleanane derivatives by the elimination of the carboxyl function at $C_{(28)}$. Consequently randialic acid B in an α,γ-unsaturated acid and therefore on hydrolysis randialic acid acetate is readily decarboxylated forming a diene of type XXII.

The mass spectra of randialic acid B and its derivatives and of the methyl ester of randialic acid A are characterized by the presence of an intense peak corresponding to the retrodiene cleavage of Δ^{12}-ursane or oleanane (XXIII) (Scheme 13). The ion XXIII readily loses its carboxyl function with the formation of an ion giving the main peak (XXIV) in all these spectra, which shows the location of the carboxyl function at $C_{(28)}$.



Scheme 13

The spectra show that the other hydroxyl group in randialic acid A and the double bond in randialic acid B, which is probably formed by the elimination of this hydroxyl group, are located in the same part of the molecule. In the NMR spectrum of the methyl ester of randialic acid B, one of the methyl groups appears at τ 8.31 and, consequently, it is present on a double bond, while in the spectrum of the methyl ester of randialic acid A the signals of the methyl group appears at τ 8.69 because the carbon atom bearing this methyl group also has an oxygen function. This fact is confirmed by the production of two isomeric acetates $C_{33}H_{50}O_4$ by the acetylation of the methyl ester of randialic acid A $C_{31}H_{50}O_4$. One of these acetates proved to be the acetate of the methyl ester of randialic acid B and the other the acetate of the methyl ester of tomentosolic acid. For tomentosolic acid, isolated together with vanguerolic acid from Vangueria tomentose, Barton et al. [17] have proposed the alternative structures XXV and XXVI (Scheme 14).

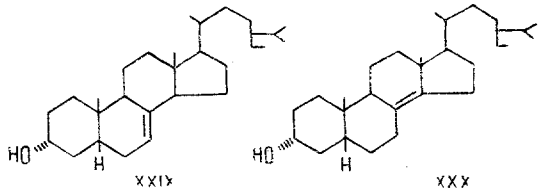


Scheme 14

Randialic acid A has the structure XXVII and randialic acid B XXVIII. The latter is isomeric with vanguerolic acid, the difference consisting in the stereochemistry at $C_{(20)}$. Thus, randialic acid A is 19-hydroxyursolic acid and randialic acid B is 19-dehydroursolic acid. The proposed structures have been confirmed by the identification of the product of the decarboxylation of the acetate of randialic acid B with the acetate of norursa-12,18(17)-dien-3β-ol.

Steroids

From the leaves of Daemia extensa [18] we have isolated only two sterols, 5β-stigmast-7(8)-en-3α-ol (XXIX) and 5β-stigmast-8(14)-en-3α-ol (**XXX**).

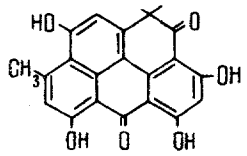


XXIX XXX

The corresponding derivatives of ergostane have been identified in extracts of Ophiorrhiza mungos [19]. In spite of the fact that both sterols have been described in the literature, they have not previously been obtained from natural sources.

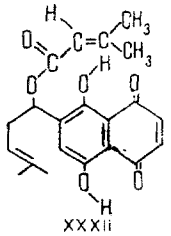
Other Polycyclic Compounds

Antibiotic X-340. The antibiotic X-340, isolated from a Streptomyces species in the process of screening soil for the isolation of organisms producing antibiotics is a compound with a polycyclic structure. The substance is very stable and is a powerful antibiotic against a broad spectrum of gram-positive organisms, including M. tuberculosis. Unfortunately, it is very feebly absorbed in the organism and is therefore not used clinically. The structure of the antibiotic obtained (**XXXI**) has been established by an X-ray crystallographic study of its dibromotetramethyl derivative. These investigations were carried out in cooperation with the chemical faculty of Sheffield University. The antibiotic X-340 is identical with the antibiotic resistomycin isolated from various Streptomyces species (West Germany [22]).



XXXI

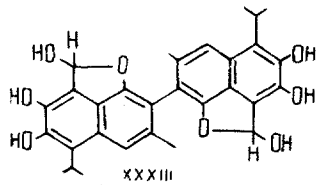
Arnebia nobilis. In a study of Arnebia nobilis it was established that an extract of the bark of the roots exhibits antibacterial and antifungal activity. We have obtained the active substance of this mixture in the form of a dark red crystalline product which has been called arnabin. It proved to be the β,β-dimethylacrylate of alkannin (**XXXII**) [23].



XXXII

This compound possesses the property of healing flesh.

Thespesia populens. Thespesin is an optically active form of gossypol **XXXIII**, the well-known optically inactive pigment from cotton seeds [24].

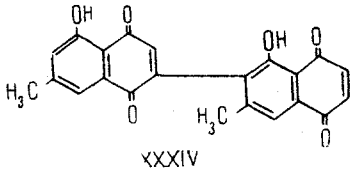


XXXIII

This compound is interesting because its optical activity is due to the hindered rotation of two naphthalene residues around the linking C—C bond (atropoisomerism).

Prof. Seshadri has also studied this compound and has independently come to the same conclusion concerning its structure [25].

Disopyros montana. Another 2,2'-binaphthalene derivative that we have characterized is diospyrin, a colored substance that we isolated from the bark of the stem of Diospyros montana. Diospyrin proved to be a 2,2'-bi[hydroxy(methyl)-naphthoquinone] [26]. For it Dr. G. S. Sidhu and his colleagues in the Regional Scientific Research Institute (Hyderabad) have established structure XXXIV [27].

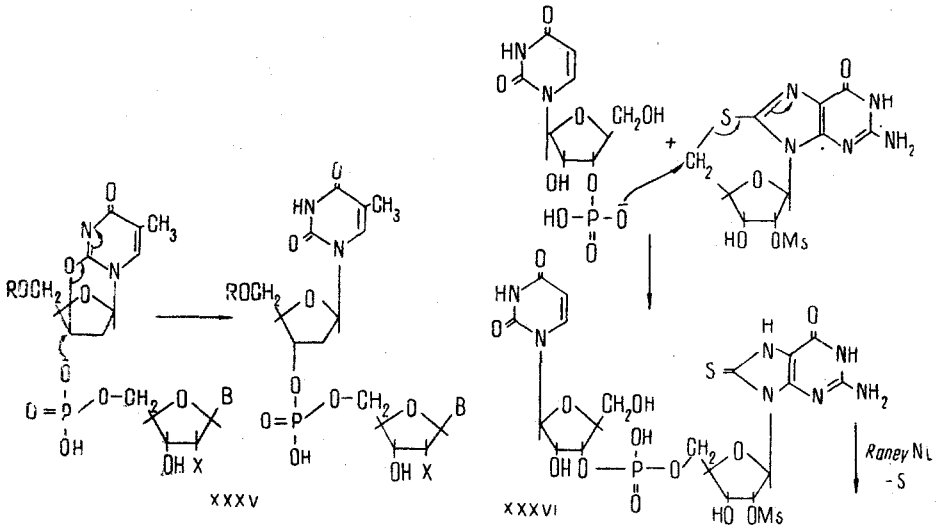


XXXIV

Nucleic Acids

Our investigations of nucleic acids of natural origin have been limited to the study of the infectious nucleic acids of two viruses. From the virus causing Ranikhet disease (a modified myxovirus of Newcastle disease) we have isolated the infectious RNA by phenolic extraction [28]. Our investigations have shown that the infectivity is retained when the RNA is coated with a lipid obtained from the virus or with lipids of animal origin, for example from mouse brain. The infectious RNA coated with lipid is not hydrolyzed by ribonuclease and withstands changes in the medium (dilution) without loss of infectivity. From Vaccinia virus (smallpox group) we have isolated two DNAs [29]. The infectious DNA comprising about 10% of the total virus DNA was readily obtained by phenolic extraction and proved to be single-stranded. The other DNA, with a double helix, required more vigorous treatment for its isolation and was never infectious.

A new method of creating the internucleotide phosphate bond using anhydropyrimidine and anhydropurine nucleosides has been developed [30, 31]. The anhydroderivatives were selected for this purpose since they have a reduced electron density and should readily react with such nucleophiles as the nucleoside phosphate anions **XXXV** and **XXXVII**. In practice, however, the reaction takes place with a good yield (50–85%) only at elevated temperatures, so that the use of this method is limited to the synthesis of dinucleotides.

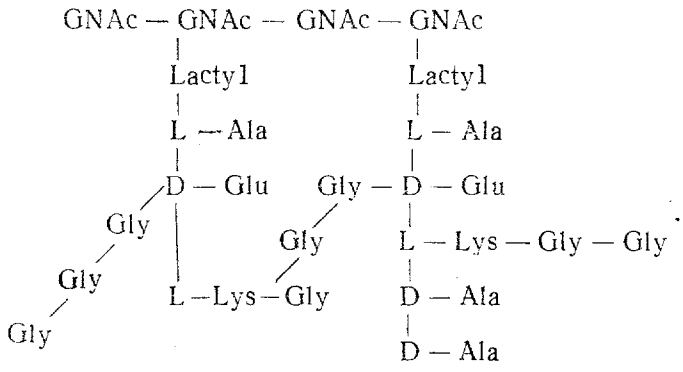


XXXV XXXVI

Other authors have recently reported similar syntheses using anhydropyrimidine nucleosides [32, 33].

Peptides

Synthesis of the peptide of the bacterial cell. It is known that the glycopeptides of the membranes of the cells of gram-positive bacteria consist of glycopeptides formed from N-acetylglucosamine, N-acetylmuramic acid, L-alanine, D-glutamic acid, L-lysine or meso-diaminopimelic acid, D-alanine, and glycine



XXXVII

A polymer of N-acetylglucosamine and N-acetylmuramic acid forms the skeleton, and the carboxyl groups of the residues of the muramic acid add to the terminal amino groups of peptide chains which, in their turn, are linked in criss-cross fashion through the amino groups of lysine residues and the α-carbonyl function of D-glutamic acid (XXXVII). This glycopeptide may then be complexed with teichoic acid or polysaccharides. The formation of the glycopeptide takes place by the stepwise addition of amino acids to uridine diphosphate N-acetylmuramic acid, and such antibiotics as penicillin, oxamycin, vancomycin, novobiocin, and bacitracin, which inhibit synthesis in the cell membrane, likewise act by blocking some particular enzymatic reaction taking part in the formation of the glycopeptides.

The synthesis of the octapeptide N-alanyl-D-glutamyl-L-lysyl-D-alanyl-D-glutamyl-L-lysyl-D-alanyl-D-alanine was first undertaken to show that the second amino acid (D-glutamic acid) was attached not through the α- but through the γ-carboxyl function [34]. This investigation was then developed to lead to the synthesis of the glycopeptide of the direct peptidization of the ε-amino group of lysine at room temperature in aqueous dioxane or ethanol. At this stage the reaction of lysine with an activated ester—for example, a p-nitrophenyl ester—is required. Thus, in this reaction there is no need for any kind of protection of the α-amino and α-carboxyl functions.

Another group of workers has reported a synthesis with the same aim but a different route [35a].

Subsequently, some analogs of the natural glycopeptides were synthesized in the hope that they would inhibit the synthesis of the cell membrane [36].

Synthesis of a lysozyme substitute. X-ray structural investigations of the lysozyme of hens'-egg white of the inhibiting complex of lysozyme performed by Phillips et al. [37] have enabled the location of all 129 amino acid residues of this enzyme to be established and the amino acids involved in the active center to be determined. As a result, the mechanism of the action of the enzyme on β(1-4)-glucosaminidase has been determined.

The main feature of this mechanism is the protonation of the glycosidic oxygen atom at the point of cleavage of the polysaccharide chain by the un-ionized γ-carboxyl group of glutamic acid residue 35 and the acetylation of the carbonium ion obtained through the rupture of the bond by the carboxyl ion of aspartic acid residue 52. This is possible when the glutamic acid residue 35 and the aspartic residue 52 are located in the hydrophobic and hydrophilic parts of the enzyme. Consequently, my colleagues have synthesized peptides with carboxyl groups localized both in the hydrophilic and in the hydroprobic parts and have found that these peptides do actually decompose the cell membrane of Micrococcus lysodeikticus in the same way as egg-white protein [38].

To synthesize these peptides, the N-carboxyanhydride of a hydrophobic amino acids (L-phenylalanine) was copolymerized with γ-benzyl L-glutamate N-carboxyanhydride in two stages. In the first stage, the N-carboxyanhydride of the hydrophobic amino acid was taken in excess, and in the second stage, which was performed without interrupting the polymerization, the glutamic acid derivative was in excess. Subsequent elimination of the benzyl group freed the carboxyl function both in the hydrophobic and in the hydrophilic parts of the polypeptides synthesized.

We have recently established that the same degree of lysozyme activity is observed in some copolymers containing an excess of a hydrophobic acid. For example, a copolymer of L-phenylalanine and L-glutamic acid (9:1) possesses a strong lysozyme activity.

The lysozyme activity was determined from the capacity of these peptides for dissolving a bacterial suspension and degrading the cell membrane with the liberation of reducing sugars. The glycopeptides formed under the action of these peptides on the cell membrane were compared with the glycopeptide obtained from the action of egg-white lysozyme. These two substances were indistinguishable on paper electrophoresis, and on hydrolysis they gave the same amino acids.

Since all these synthetic peptides were insoluble in water, they were used in the form of a finely dispersed suspension A suspension containing 3 mg/ml of the polymer of phenylalanine and glutamic acid (9:1) had the same order of activity as a solution of crystalline lysozyme (0.1 mg/ml).

The insolubility of these polymers makes it difficult to determine their biological activity. This is the first case of the synthesis of an enzyme substitute with a considerable degree of activity.

This short sketch has outlined only characteristic fragments of the work in various fields of the chemistry of natural substances carried out in the Central Drug Research Institute at Lucknow (India).

REFERENCES

1. S. Bhattacharji, V. N. Sharma, and M. L. Dhar. J. Sci. Industr. Res., **15B**, 363, 1956.
2. S. Bhattacharji and M. L. Dhar, Proc. Internat. Symp. Med. Plants, held under the auspices of UNESCO at Kandy, Ceylon, 109, 1964.
3. S. N. Pradhan, Proc. Internat. Symp. Med. Plants, held under the auspices of UNESCO at Kandy, Ceylon, 203, 1964.
4. A. K. Bhatnagar and S. P. Popli, Indian J. Chem., **5**, 102, 1967.
5. A. K. Bhatnagar, S. Bhattacharji, A. C. Roy, S. P. Popli, and M. L. Dhar, J. Org. Chem., **32**, 819, 1967.
6. A. K. Bhatnagar and S. P. Popli, Exper., **23**, 242, 1967.
7. F. Anwar, S. P. Popli, R. M. Srivastava, and M. P. Khare. Exper., **24**, 992, 1968.
8. M. Tomita, M. Koznka, and Sheng-Tehlu, Chem. Abs., 67, 3124, 1967.
9. S. N. Pradhan, K. Pandey, and R. P. Badola, Brit. J. Anaesthesia, **36**, 604, 1964.
10. T. R. Rajagopalan, S. Bhattacharji, and M. L. Dhar. Symp. on Drugs and Antibiotics at the Defence Science Laboratory, Ranpur, 121, 1961.
11. A. K. Bhatnagar, S. Bhattacharji, and S. P. Popli, Indian J. Chem., **3**, 525, 1965.
12. A. R. Battersby, R. S. Kapil, D. S. Bhakuni, S. P. Popli, J. R. Merchans, and S. S. Salgar, Tetrah. Lett. 4965, 1966.
13. D. S. Bhakuni and M. M. Dhar, Exper., **24**, 1024, 1968.
14. N. Chatterji, R. P. Rastogi and M. L. Dhar, Indian J. Chem., **1**, 212, 1963.
15. N. Chatterji, R. P. Rastogi, and M. L. Dhar, Indian. J. Chem., **3**, 24, 1965.
16. J. S. Tandon, K. P. Agarwal, and M. M. Dhar. Indian J. Chem., **4**, 483, 1966.
17. D. H. R. Barton and C. J. W. Brooks, J. Chem. Soc., 257, 1951.
18. S. Rakhit, M. M. Dhar, N. Anand, and M. L. Dhar, J. Sci. Industr. Res., 18B, 422, 1959.
19. K. P. Agarwal and M. M. Dhar, J. Sci. Industr. Res., 16C, 114, 1959.
20. V. C. Vora, K. Shete, and M. M. Dhar, J. Sci. Industr. Res., **16C**, 182, 1957.
21. N. A. Bailey, C. P. Falshaw, W. D. Ollis, M. Watanabe, M. M. Dhar, A. W. Khan, and V. C. Vora, Chem. Comm., 374, 1968.
22. H. Brockman and G. Schmidt-Kastner, Chem. Ber., **87**, 1460, 1954.
23. Y. N. Shukla, J. S. Tandon, D. S. Bhakuni, and M. M. Dhar, Exper., **25**, 1969.
24. D. S. Bhakuni, M. M. Dhar, and V. N. Sharma. Exper., **24**, 109, 1968.
25. S. C. Datta, V. V. S. Murti, and T. R. Seshadri, Current Science, **37**, 135, 1968.
26. R. S. Kapil, M. M. Dhar, J. Sci. Industr. Res., 20B, 498, 1961.
27. G. S. Sidhu and M. Pardhasardhi, Tetrah. Lett., 1313, 1967.
28. M. M. Dhar and O. P. Babbar, Exper., **19**, 100, 1963.
29. O. P. Babbar, B. L. Chowdhury, and M. M. Dhar, Indian. J. Biochem., **1**, 83, 1964.
30. K. L. Agarwal and M. M. Dhar, Tetrah. Lett. 2451, 1965.
31. P. C. Srivastava, K. L. Nagpal, and M. M. Dhar, Exper., **24**, 657, 1968.
32. J. Zemlika and J. Smrt. Tetrah. Lett. 2083, 1964.
33. J. Nagyvary and J. S. Roth. Tetrah. Lett. 617, 1965.
34. M. C. Khosla, N. C. Chaturvedi, H. C. Garg, and N. Anand, Indian J. Chem., **3**, 111, 1965.
35. M. C. Khosla, N. C. Chaturvedi, and N. Anand, Indian J. Chem. **5**, 237, 1967.
35a. A. E. Lanzilotti, E. Benz, and L. J. Goldman, Amer. Chem. Soc., **86**, 1880, 1964.
36. N. C. Chaturvedi, M. C. Khosla, and N. Anand, J. Med. Chem., **9**, 971, 1966.
37. C. H. Blake, D. F. Koeng, G. A. Mair, A. North, D. C. Phillips, and V. R. Sarma, Nature **206**, 757, 1965.
38. V. K. Naithani and M. M. Dhar. Biochem. Biophys. Res. Comm., **29**, 368, 1967.

25 September 1968

Central Institute for Medicinal Plants, Lucknow, India