Studies on the Chemistry of Lichens, XIX. New Amino Compounds from Anaptychia fusca and Several Stereocaulon Species

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The lichen Anaptychia fusca and several Stereocaulon species have been chemically investigated with regard to their content of unknown ninhydrin-positive compounds. New aliphatic and alicyclic, amino components isolated from water extracts of these lichen species have been characterised as aminopentitol, aminohexitol and aminocyclitetrol. To our knowledge, this is the first time that compounds such as these have been found in lichen material. Aminopentitol and aminocyclitetrol appear to be characteristic constituents of Anaptychia fusca, whereas aminohexitol has been found in several species of Stereocaulaceae.

Introduction

Owing to the discovery in recent years of a range of unusual aminosugars, frequently occurring as components of microbial metabolites, there is much current interest in the chemistry of this class of compounds. The isolation of a new compound from the lichen species Xanthoria parietina, established as 3-hydroxy-4,4-aminohydroxymethyl)tetrahydrofuranyl alcohol, C₃H₃NO₅, has recently been reported from our institute [1]. However, this constituent may also be considered as 5-amino-1,5-dideoxy-glycopyranose.

In a more comprehensive investigation of the free sugars and ninhydrin-positive compounds of several Norwegian lichen species [2], two unusual substances were observed in the species Anaptychia fusca and Stereocaulon pascale. Both compounds, which afforded typical colours after reaction in buffer solution with ninhydrin, were described by column chromatographic methods, but not identified. The purpose of the present paper is to describe the isolation and identification of these compounds, also to offer evidence in favour of their proposed formulation as aminopentitol and aminocyclitetrol (a mixture designated as Anaptychia-X) from Anaptychia fusca and aminohexitol (as Stereocaulon-X) from Stereocaulon species.

The isolated substances belong to the class of acyclic and alicyclic amino polyols. Cyclitols bearing amino or substituted amino groups in place of hydroxyl groups, the so-called inosamines, have been revealed as constituents of several antibiotics. As yet no inosamines have been found in nature in their free state and the most frequently encountered natural inosamines are bonded in antibiotics.

The isolated aminocyclitetrol (dideoxyinosamine) from Anaptychia fusca in this investigation has a configuration unprecedented in natural inositols. It is not rare to find more than one polyol in the same species and several cases exist where polyols of different configuration co-exist, even in the same species. The discovery of the new amino compounds in this investigation should stimulate much work concerning the still unknown compounds 2, 5, 6 and 12 as described in our report of 1970 on other lichen species [2].

The identification of the compounds is based on elementary analyses, IR, NMR and mass spectrometry. The new amino compounds have not yet been synthesised and their structure is not, therefore, fully established.

Experimental

Materials and isolation of the amino compounds.

The main part of the species Anaptychia fusca (Huds.) Vain. (in all 5.54 kg) used in this investigation was collected on the island of Runde in July-August, 1968–76. Species of Stereocaulaceae (in all 9.31 kg) were collected mainly in Folldal during 1975–77. The lichen materials were cleansed of all extraneous plant materials, air-dried and pulverised. The materials were at first treated with chloroform, acetone and ethanol, and thereafter extracted using the solvent mixture of water-isopropanol-0.05 N HCl...
(8 + 1 + 1) by continuously shaking for 20 h. The water extract was centrifuged, filtered and evaporated to a smaller volume. Absolute ethanol was then applied to the cloudy solutions increasing the volume by 3–4 times in order to remove polysaccharides and proteins, treated with activated charcoal (Darco G 60) and filter aid, and the filtrates once more reduced to small volumes. The pH of the concentrated extracts, which by this time were quite clear and colourless, were regulated to approx. 7–8 and passed through columns (290x65 cm) of cation exchange resin (Dowex 50 W, X8, 50–100 mesh, H⁺). After washing with water the amino acids and other amino compounds were displaced from the columns with 2 N ammonia. The ammonia eluates were evaporated to dryness and traces of NH₃ removed in vacuum. The residues were then taken up in a solution of ammoniumacetate (0.0875 N, pH 8.3) and treated with the Amberlite exchange resin IRC 50, 22–45 mesh, in the NH₄ form. After removal of acidic and neutral amino acids by eluting with the ammoniumacetate solution, the basic unknowns Anaptychia-X and Stereocaulon-X, together with small amounts of lysine, histidine and arginine, were collected in the am­ monia eluate (0.5 N). The ammoniacal solutions were evaporated to syrups which were redissolved in 50 ml sodium citrate buffer (0.2 m, pH 4.35) and finally filtered through a Millipore Filter (0.45 µm).

To obtain a sufficient quantity of Anaptychia-X and Stereocaulon-X from the purified citrate solutions for detailed examinations, orders of separations, 1490 and 690 respectively, were performed by ion-exchange chromatography on columns (190x9 mm I.D.) equipped with type Durrum high efficiency cation exchange resin (Pierce DC-6A). The column effluent was pumped at a rate of 40 ml/h and the column temperatures used were 50 and 40 °C for the isolation of Anaptychia-X and Stereocaulon-X respectively. The composition of the trisodium citrate buffers used was 0.35 m with respect to Na⁺ (pH 5.28) and 0.38 m (pH 4.25) for the separation of the above fractions respectively. Aliquots (40 µl) of the extracts were transferred to the columns and washed in with a pH 2.2 citrate buffer (0.2 m Na⁺). Fractions (11 ml) were collected automatically by means of an amino acid analyser and an aliquot (0.5 ml) of every fourth fraction was analysed for Anaptychia-X and Stereocaulon-X with the ninhydrine reagent. The eluates were later on applied to freshly regenerated columns of Dowex 50 W, X8, 50–100 mesh, H⁺, in order to remove the sodium citrate. Anaptychia-X crystallised by storage in a desiccator over P₂O₅ and solid NaOH.

Analytical methods. Acetylation of Anaptychia-X and Stereocaulon-X were performed using acetic acid anhydride in dry pyridine (1+3) and refluxing for 30 min. The periodate oxidation of Anaptychia-X was carried out in darkness at 4 °C in a 0.2 m NaHCO₃ solution of 0.17 m NaIO₄ for 23 h. In these conditions the malondialdehyde obtained did not oxidise further in spite of the active methylene group hydrons. The aldehyde was isolated as 2,4-dinitrophenylhydrazone.

Glucos- and mannosaminitol were prepared in our laboratory from the corresponding aminosugars by NaBH₄-reduction, and 1-amino-1-deoxyarabinitol and aminopentahydroxy cyclohexane (aminocyclipentol) using the methods of Winsstock and Piaut [3] and Grosheintz and Fischer [4] respectively. Other standards were purchased from the commercial sources and were of guaranteed grade purity.

Using TLC technique on cellulose plates (MN 300) with the elution agent ethanol/amyl alcohol/ammonia/water (62 + 15 + 15 + 8), the presence of two components was observed in the fraction Anaptychia-X, both of which afforded typical colours with ninhydrine and dimethylaminocinamic aldehyde. The Rf-values of the consisting two components of Anaptychia-X were 0.38 and 0.71; fructosamine (ref. stand.) 0.58.

The elemental analyses were carried out by Analytische Laboratorien, Elbach-BRD. The high resolution mass spectra (HRMS) has been obtained from the Technical University of Trondheim and Shrader Analytical Laboratories, Detroit-USA. Trimethylsilyl (TMS) derivatives were prepared rapidly with trimethylchlorosilane and analysed by HRMS. Proton nuclear resonance (NMR) spectra in DMSO-d₆ at frequency of 60 Mc.p.s. Peak positions are given in δ-values (ppm) referenced internally downfield from tetramethylsilane. Infrared (IR) spectra were obtained from KBr discs.

All concentration steps were carried out at reduced pressure (water aspirator) using a rotary evaporator operating at temperatures not exceeding 35 °C.

Acknowledgements

The major of the Stereocaulaceae species investigated in this work has been collected and identified
Results and Discussion

The purity of the isolated fractions Anaptychia-X and Stereocaulon-X used for the present investigations was confirmed by column chromatography on an amino acid analyser and by TLC. The position occupied by the elution peak corresponded to that determined previously [2], and only a single, symmetrical peak could be observed on the column chromatograms. Analyses by use of different column, buffer and temperature conditions on the amino acid analyser and by comparison with reference compounds, displayed that the fractions Anaptychia-X and Stereocaulon-X were neither identical with any of the following compounds; glucos-, mannos-, galactos-, fructos- or allosamine, glucos- and mannos-aminitol; nor with aminopentahydroxy cyclohexane. The mentioned compounds all exhibited short retention times but were not identical to those of the isolated compounds. Anaptychia-X emerged from the column with a retention time close to that of arabino-amylosaminitol, whereas the less basic compound Stereocaulon-X left the column almost at the same moment as mannosaminitol.

The ratio of the areas under the 440 nm and 570 nm absorption peak tracing for 11 standard amino acids, amino sugars and aminoaalditols, was average found to 0.14, for Stereocaulon-X 0.14 and for Anaptychia-X 0.29. On the basis of the work published by Conkerton et al. [5] the high ratio value found for Anaptychia-X confirms the TLC analysis that this fraction actually is a mixture of two ninhydrin-positive components, henceforth designated as Anaptychia-X1 and X2. On the same reasoning it is assumed that Stereocaulon-X is a single component, a fact which was later confirmed.

Since the nitrogen atom is basic in the compounds themselves and reacts with ninhydrine, it must in all three cases be present as primary amino groups.

One of the most significant features is that the compounds Anaptychia-X1 and X2 isolated from Anaptychia fusca have not so far been found in extracts from 45 other lichen species examined in the years 1970–79 at our institute. This supports our remark in 1970 [2] that Anaptychia-X are specific components of the mentioned species. Xantholamine found in Xanthoria parietina [1] has also remained undetected in other species. The compound Stereocaulon-X has only been detected in the family Stereocaulaceae, and hitherto in the species S. alpinum Laur., S. botryosum Ach. em. Frey, S. coniophyllum Lamb, S. condensatum Hoffm., S. dactylophyllum Flörke, S. delisei Bory ex Duby, S. evolutum Graewe, S. glareosum (Sav.) Magn., S. grande (Magn.) Degel., S. nanodes Tuck., S. pascale (L.) Fr., S. pileatum Ach., S. rivulorum Magn., S. saxatile Magn., S. spatuliferum Vain., S. symphyceilum Lamb and S. tomentosum Fr. The species S. articum Lyne, S. vesuvianum Pers. and S. groenlandicum (Dahl) Lamb have also been investigated at our institute, but the presence of this component was rather uncertain.

Anaptychia-X

It was impossible to separate the two components X1 and X2 in the mixture isolated from Anaptychia fusca. However, it was expedient to carry out investigations on the mixture and this sufficed to unravel the structure of the components. The fraction Anaptychia-X is readily soluble in water and gives evolution of nitrogen when treated with nitrous acid. Unsaturation was not observed in the IR spectra, nor was hydrogen absorbed by hydrogenation. Neither elementary analyses, NMR, or IR indicated the presence of CH3-groups bonded to carbon, nitrogen oxygen atoms.

The IR spectrum of Anaptychia-X showed alkyl group bands at 2900 and 1450 cm−1, broad 3340 cm−1 band due to OH and NH stretchings, 1575 cm−1 (amide band), 1060 cm−1 (alcohol band), and 815 cm−1 due to out of plane band for NH group.

Chemical ionisation (isobutane) mass spectrum exhibited two important peaks at m/e 152 (R.I. 100%) and m/e 164 (R.I. 63%) corresponding to the molecular ions of the two components Anaptychia-X1 and X2 respectively. Metastable ions are measured at m/e 130.0, 118.0 and 98.5 associated with the transitions m/e 154 → m/e 146, m/e 152 → m/e 134, and m/e 132 → m/e 114 respectively. In the E.I.MS the most abundant ions were seen at m/e 145 (C6H5NO), 133 (C6H11NO3), 132 (C6H10NO3, 100%), 120 (C6H10NO3), 114 (C6H7NO2), 102 (C6H4NO2), 90
(C\textsubscript{4}H\textsubscript{8}N\textsubscript{0} 2), 73 (C\textsubscript{3}H\textsubscript{2}NO or C\textsubscript{3}H\textsubscript{2}O), 72 (C\textsubscript{3}H\textsubscript{2}NO) and 60 (C\textsubscript{2}H\textsubscript{6}NO, 100%), fragments which are quite common in MS of polyols. On the basis of HRMS the elementary composition of the molecular ions was found to be C\textsubscript{12}H\textsubscript{2}NO\textsubscript{3} (m/e 151) and C\textsubscript{14}H\textsubscript{2}NO\textsubscript{4} (m/e 163), and the other ions at m/e 132, 120, 102, 90, 73, 72 and 60 could easily be explained as m/e 163 - CH\textsubscript{2}OH, m/e 151 - CH\textsubscript{2}OH or m/e 163 - CH(OH)CH\textsubscript{2}OH, m/e 151 - CH(\textsubscript{2}OH)CH\textsubscript{2}OH, H\textsubscript{2}N - CH\textsubscript{2} - CH - CH\textsubscript{2}, H\textsubscript{2}N - CH\textsubscript{2} - CH - CH\textsubscript{2} and H\textsubscript{2}N = CH - CH - CH\textsubscript{2}, respectively. The trimethylsilyl ether derivatives of Anaptychia-X1 (MW 511, C\textsubscript{16}H\textsubscript{3}NO\textsubscript{5}Si\textsubscript{3}) and Anaptychia-X2 (MW 523, C\textsubscript{18}H\textsubscript{3}NO\textsubscript{6}Si\textsubscript{3}) exhibited no molecular peaks in the mass spectrum. Two important peaks in the high mass range of a HRMS, however, were found at m/e 348 (93%) C\textsubscript{15}H\textsubscript{3}NO\textsubscript{5}Si\textsubscript{3} and m/e 336 (7%) C\textsubscript{14}H\textsubscript{3}NO\textsubscript{5}Si\textsubscript{3} corresponding to the fragments (m/e 523 - CHO\textsubscript{3}SiMe\textsubscript{3} - SiMe\textsubscript{3}) and (m/e 511 - CHO\textsubscript{3}SiMe\textsubscript{3} - SiMe\textsubscript{3}).

The above evidence, combined with the elemental analyses of the acetylated mixture (Table I), permitted the unequivocal conclusion that Anaptychia-X consists of the two components aminopentitol (C\textsubscript{15}H\textsubscript{2}NO\textsubscript{3}) and aminocyclitetrol (C\textsubscript{16}H\textsubscript{3}NO\textsubscript{3}).

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Table I. Elemental analysis of the acetylated mixture isolated from Anaptychia fusca. MW measured osmometric in chloroform and acetone.

<table>
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<tr>
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<th>% C</th>
<th>% H</th>
<th>% N</th>
<th>% O</th>
<th>% NH</th>
<th>MW</th>
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<td>Observed</td>
<td>49.75</td>
<td>6.32</td>
<td>3.80</td>
<td>39.87</td>
<td>3.9</td>
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<tr>
<td>Calculated when equal amounts of C\textsubscript{15}H\textsubscript{3}NO\textsubscript{5} and C\textsubscript{16}H\textsubscript{3}NO\textsubscript{6}</td>
<td>50.67</td>
<td>6.31</td>
<td>3.82</td>
<td>39.21</td>
<td>4.1</td>
<td>367</td>
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the fact that it is an equal amount of the two components XI and X2.

**Stereocaulon-X**

The fraction was isolated in syrupy condition, and series of analyses unambiguously demonstrated the presence of a single component. The IR spectrum shows the expected bands at 3300, 2930, 1595, 1490, 1400 and 1035 cm$^{-1}$, characteristic to compounds belonging to the aminoalditols. No bands could be seen between 1400 and 1350 cm$^{-1}$, and no O-, C-, or N-CH$_3$ groups were present.

Chemical ionisation mass spectrometry of Stereocaulon-X showed base peak at $m/e$ 182 (M$^+$) corresponding to the component C$_6$H$_{15}$NO$_5$. Other abundant peaks in MS were observed at $m/e$ 164, 150, 146, 132, 110, 102, 90, 73, 72, 69 and 60. An identical fragmentation pattern was obtained by C.I. and E.I.MS of glucosaminitol, and HrMS results gave elementary composition of the ions identical with those given for Anaptychia-X previously shown. Metastable ions at $m/e$ 148.0, 116.2 and 86.6 are associated with the transitions $m/e$ 182 $\rightarrow m/e$ 164, $m/e$ 150 $\rightarrow m/e$ 132, and $m/e$ 150 $\rightarrow m/e$ 114 or $m/e$ 120 $\rightarrow m/e$ 102 respectively.

By acetylation the Stereocaulon-X readily formed the hexaacetate with the base peaks at $m/e$ 433 (M$^+$, C$_{18}$H$_{27}$NO$_{11}$) and $m/e$ 373 (M$^+$ - CH$_3$COOH) in a C.I.MS. Other prominent peaks were seen at $m/e$ 391, 331 and 313 responsible to the fragments M-42, M-42-60 and M-2•60 respectively. The acetate exhibited absorption frequencies at the expectant positions 1730, 1665, 1630, 1480, 1420, 1365, 1220 and 1025 cm$^{-1}$.

Fully trimethylsilylated ether of Stereocaulon-X (C$_{24}$H$_{63}$NO$_5$Si$_6$, MW 613) does not demonstrate any molecular ion in HrMS. The most important primary fragmentation is shown to be cleavage of the carbon chain by C1-C2 and subsequent loss of two mol TMS, giving the fragment C$_{14}$H$_{36}$NO$_4$Si$_3$ at $m/e$ 366 (1.4%). The formation of the $m/e$ 348 (1.7%) C$_{14}$H$_{34}$NO$_3$Si$_3$ step occurs through loss of water. The most characteristic features of the remaining peaks is the high intensity ions at $m/e$ 145 (25%) C$_9$H$_{21}$O$_2$Si and $m/e$ 132 (83%) C$_5$H$_{14}$NO for the fragments CH$-\text{CH}_2$O$\text{TMS}$ and HO$\text{CH}_2$O$\text{TMS}$ respectively.

The prominent fragment obtained at $m/e$ 307 (C$_{12}$H$_{31}$O$_2$Si$_3$), is formed by cleavage of the C3 to C4 bond and represents the fragment TMS - O = CH$\text{CH}_2$O$\text{TMS}$ and HO=CH$\text{CH}_2$O$\text{TMS}$ respectively.

The analytical work leaves no doubt that Stereocaulon-X is an **aminohexitol**, C$_6$H$_{15}$NO$_5$, with the NH$_2$ group attached to carbon atom, C5 or C6.