Tetrahedron Letters Vol. 21, pp 4387 - 4390 0040-4039/80/1101-4387$02.00/0

A NEW CYTOKININ FROM THE FRUITS OF ZANTEDESCHIA AETHIOPICA

H.J. Chaves das Neves *

INIC - Departamento de Química, Faculdade de Ciências e Tecnologia
Universidade Nova de Lisboa, Quinta do Cabeço, Olivais
1899 Lisboa Codex, Portugal

and

M. S. S. Pais

INIC - Laboratório de Botânica, Faculdade de Ciências,
Rua da Escola Politécnica, 1294 Lisboa Codex, Portugal

SUMMARY: A new purine derivative with cytokinin activity was isolated from the cuckoo-pint fruits (Zantedeschia aethiopica) and identified as 6-(o-hydroxybenzylamino)-2-methylthio-9-β-D-glucofuranosylpurine 1.

Although synthetic 6-benzylaminopurines have long been known as active cytokinins in in vitro experiments[1] and can be metabolized when exogenously supplied[2] to a variety of plants, their isolation from natural sources where they can act as endogenous hormones is very unusual. However, 6-(o-hydroxybenzylamino) -9-β-D-ribofuranosyl purine was reported to be present in mature poplar leaves[3] and more recently it could be isolated in relatively high yields from the fruits of the cuckoo-pint and fully characterized by chemical and spectroscopical methods[4]. We wish now to report the isolation and identification of a new cytokinin also present in the cuckoo-pint fruits as a minor component.

The fruits were freed from strange materials after collection, cut in pieces and extracted with 80% methanol during 48 hrs. at 1 - 3° C. After filtration, the methanolic solution was concentrated under 30° C at reduced pressure, acidified to pH 1.5 with aqueous 1 M HCl and extracted with ethyl acetate. The aqueous fraction was then neutralized with 10% aqueous solution of sodium hydroxyde and extracted with n-butanol. The collected butanolic fractions were evaporated at reduced pressure, the residue was taken with water and again acidified with aqueous 1 M HCl to pH 1.5. The acidified solution was then passed through

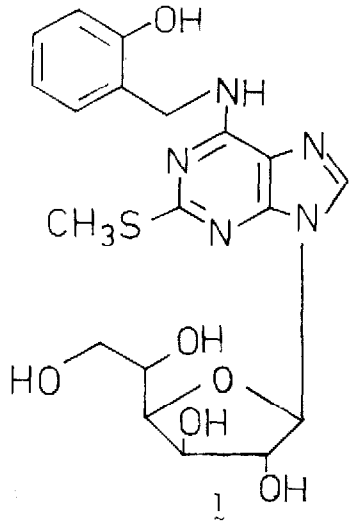
a column of a cationic ion exchange resin (Dowex 50W-X8, H^+ form). The basic fraction retained on the column was eluted with an aqueous 5M ammonium hydroxyde solution. The solvent was evaporated at reduced pressure and the residue was further purified by reverse phase chromatography through a column of Sephadex LH-20 in 35% ethanol. The collected fractions were examined for cytokinin activity by means of the tobacco disks bioassay[5]. The fraction with U.V. absorption maxima at (pH 1) λ_{max} 286 nm (pH 7) λ_{max} 283 nm and (pH 12) λ_{max} 283 nm and 290 nm (sh), indicative of substitution at the N^6 and 9-positions of an aminopurine[6] was further examined.

The solid probe mass spectrum of the above fraction showed a set of ions at m/e 280, 267, 194, 178, 164, 149, 148, 136, 135, 121, 119, 108, and 81 which suggest the presence of a hexose derivative of adenine[7]. Prominent peaks at m/e 449 (M^+), 432 (M^+ - OH), 357 (M^+ - C_6H_4O), 343 (M^+ - C_7H_6O) and 328 (M^+ - C_7H_8NO) indicate the presence of a hydroxybenzylamino group at the 6-position of the purine nucleus. This is confirmed by the presence of fragment ions at m/e 122 (C_7H_8NO), 107 (C_7H_7O) and 93 (C_6H_5O). A fragment at m/e 433 probably arises from rearrangement of the M^+ with loss of NH_2. Other significant ions appear at m/e 326, 313, 299, 295, 279, 254, 239, 238, 224, 210, 195, 192, 181, 151, 92, 91, 77, and 58. It is interesting to note that many of these fragments correspond to the ones shown by hexose derivatives of adenine shifted 46 a.m.u. higher. This suggests the presence of a methylthio group in agreement with the U.V. data[8], which is confirmed by the signal at 2.51 p.p.m. appearing in the PMR spectrum (60 MHz, DMSO-d_6) of the compound. The position of this substituent is probably at C-2 of the purine nucleus which is in agreement with the one proton signal at 8.27 p.p.m. corresponding to the adenine C-8 proton[8]. The fragments at m/e 313, 181 and 151 fully corroborate this conclusion[9].

Purity of the compound was tested by g.c.m.s. after silylation with BSTFA carried out in a screw-cap teflon-lined derivatization vial at 120° C for 1 hr. The mass spectrum of the TMS derivative is quite instructive. The presence of a molecular ion at m/e 809 corresponds to the introduction of five TMS groups as it would be expected from the above considerations. The presence of a hexose moiety is indicated by the fragments at m/e 319, 217, 204, 169, 147, 129, 103, and 73 present in most spectra of carbohydrate TMS derivatives, together with the ions at m/e 451, 361, and 271 characteristic of per-TMS derivatives of purine 9-glucosides[10].

An interesting point respects to the configuration of the hexose moiety. Comparison of the relative intensities of the peaks at m/e 204, 217 and 319 in the mass spectrum of the TMS derivative, indicates that the hexose is in the furanose form rather than the pyranose. The ion at m/e 217 is significantly more in-

tense than m/e 204 while the ion at m/e 319 is a relatively intense peak. This fragmentation pattern is characteristic of glucofuranosyl derivatives[11]. The peaks at m/e 604 (M^+ - 205) and m/e 439 [(M^+ - 165) - 205] further support the furanosyl structure. As a matter of fact the loss of 205 a.m.u. results from elimination of the TMS 5 and 6 carbons from a furanose configuration rather than from a pyranose [11]. The absence of an ion at 677 (M^+ - 132) also supports this conclusion.



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Identification of the hexose substituent was made by analysis of the hydrolysis products. The isolated product from the fruits was treated with 2 M HCl in a screw-cap derivatization vial for 2 hrs. at 110° C. The solution was evaporated to dryness under a stream of nitrogen and the residue was treated with BSTFA. The TMS derivatives thus obtained were subjected to gas chromatography[12]. The chromatogram showed peaks with identical retention times as the ones shown by a glucose control and were undistinguishable from these on coinjection. This result was confirmed by g.c.m.s. experiments[11]. Another peak in the chromatogram was shown to be identical on coinjection to a TMS derivative of a sample of authentic 6-(o-hydroxybenzylamino)-2-methylthiopurine, synthesized from 2,6-bis-(methylthio)purine and the appropriate amine[8]. Although the stereochemistry of the glucosidic linkage could not yet be unequivocally established, analogy to other known cytokinin metabolites[13] makes the hypothesis of a β-linkage very likely. Thus, it is our conclusion that the structure of the cytokinin active compound isolated from the fruits of Zantedeschia aethiopica we are dealing with corresponds to 6-(o-hydroxybenzylamino)-2-methylthio-9-β-D-glucofuranosylpurine 1.

It is no uncommon fact that purine bases with cytokinin activity are isolated from vegetal materials together with their methylthio analogues[14]. The

fact that 6-benzylaminopurine is known to be metabolized by soybean callus tissue to give a stable glucofuranosyl derivative arises the question of knowing whether 1 represents an active cytokinin acting as endogenous hormone or rather a storage form.

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Acknowledgements

The authors wish to thank G.J. Nicholson of the Institut fuer Organische Chemie of the University of Tuebingen (G.F.R.) for the g.c.m.s. and the Instituto Nacional de Investigação Científica e Tecnológica for financial support.

(Received in UK 22 August 1980)