XANTHOMEGNIN AND VIOMELLEIN DERIVATIVES FROM SUBMERGED CULTURES OF THE ASCOMYCETE *Nannizzia cajetani*

Petr SEDMERA[a], Jindřich VOLC[a], Jan WEIJER[b], Jindřich VOKOUN[a] and Vladimír MUSÍLEK[a]

[a] *Institute of Microbiology, Czechoslovak Academy of Sciences, 142 20 Prague, Czechoslovakia and*
[b] *Department of Genetics, University of Alberta, Edmonton, Canada T6G 2E9*

Received July 31st, 1980

Antibacterial metabolites isolated from a submerged culture of the ascomycete *Nannizzia cajetani* AJELLO grown on wort medium were spectroscopically identified as dimeric naphthoquinone derivatives xanthomegnin (*I*), viomellein (*II*), 3,4-dehydroxanthomegnin (*III*) and two, not previously described pigments: 3',4'-dehydroviomellein (*IV*) and 3,4,3',4'-bisdehydroxanthomegnin (*V*).

Nannizzia cajetani AJELLO[1,2] represents the perfect form of the keratinophilic fungus *Microsporum cookei* AJELLO[3]. Reports[4] indicate that so far only constituents of the conidial stage (*M. cookei*) have been investigated. Among five chromatographically characterized pigments from the chloroform extract, one was identified as xanthomegnin (*I*), the other unidentified pigments were reported as 1,4-naphthoquinones related to xanthomegnin on the basis of their IR and UV spectra[4]. The present investigation of *N. cajetani* metabolites was motivated by marked antibacterial activity of the chloroform mycelial extract which appeared to be related to the production of orange-purple red pigments. This paper deals with the identification of five isolated antibiotic substances.

The main component of the pigment mixture exhibits in its mass spectrum a cluster of ions *m/z* 578, 576 and 574 with a composition of $C_{30}H_{26}O_{12}$, $C_{30}H_{24}O_{12}$ and $C_{30}H_{22}O_{12}$. According to its ^{1}H-NMR spectrum (Table I), this compound contains a $CH_3CH(O—)CH_2$-group, one isolated aromatic proton, an aromatic methoxyl and phenolic hydroxyl group. Fifteen signals were observed in its ^{13}C-NMR spectrum (Table II). Judging from the off-resonance spectrum, ten hydrogen atoms are directly bonded to carbon atoms. These results together with the mass spectroscopic data, indicate that the compound is the symmetric dimer $C_{30}H_{22}O_{12}$. The ions *m/z* 578 and 576 should be considered as M + 4 and M + 2 ions and consequently correspond to the presence of two quinoid systems for which the reduction upon electron impact is typical[5]. Presence of the above mentioned moiety is also

confirmed by ^{13}C-NMR and IR spectra. Chemical shifts of quinone carbonyls (186·2 and 180·0 ppm) show that the former is hydrogen-bonded. The deduced structure features fit neatly the structure of a known naturally occurring naphthoquinone pigment: xanthomegnin (*I*). Comparison of ^{1}H- and ^{13}C-NMR data with literature[6] definitely confirms this identity, especially on the basis of the similarity index[7] calculated using the ^{13}C-NMR spectra.

Proton-coupled ^{13}C-NMR spectra of xanthomegnin in $CDCl_3$ (Table II) support conclusions[8] leading to the structure revision of this compound on the basis of the proton-coupled spectrum of xanthomegnin diacetate. Taking this work into account, we were able to resolve more couplings and prove some of them by using low-power selective decoupling[9] of $H_{(4)}$ and $H_{(5)}$ signals. The results allow us to differentiate between the assignments of $C_{(5a)}$ and $C_{(8)}$ since the singlet δ 134·8 must be due to $C_{(8)}$ which is the only carbon in the molecule with no protons within three bonds. The signal at 123·1 ppm (a doublet, $J = 2{\cdot}9$ Hz) is then assigned to $C_{(5a)}$; the observed splitting is caused by geminal coupling to $H_{(5)}$.

Identification of xanthomegnin facilitated the structure determination of the metabolite $C_{30}H_{24}O_{11}$ (high resolution measurement of the M+2 ion) which is second in abundance. This compound was identified as viomellein (*II*) by comparison of its ^{1}H- and ^{13}C-NMR spectra with those reported in literature[6] (similarity index[7] $S = 0{\cdot}9729$).

Also the metabolite $C_{30}H_{20}O_{12}$ (as determined by high resolution, exact mass determination of the M, M+2 and M+4 ions) is a known compound, 3,4-dehydro-

TABLE I

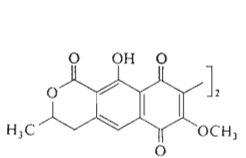
^{1}H-NMR Chemical Shifts of Compounds *I*—*IV*

Chemical shifts given in the δ-scale.

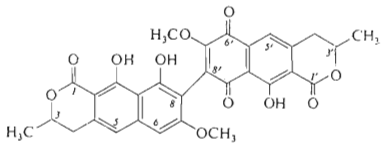
Compound	3-CH_3[a]	3-H[b]	4-H_2[c]	5-H[d]	6-H[d]	7-OCH_3[d]	9-OH[e]	10-OH[e]
I	1·56	4·68	3·05	7·49	—	4·16	—	13·17
II[f]	1·54	4·70	3·01	7·49	—	3·90	—	13·50
II[g]	1·54	4·70	3·01	6·96	6·66	3·86	9·80	13·88
III[f]	1·56	4·68	3·05	7·51	—	4·16	—	13·17
III[g]	2·35[d]	—	6·39[d]	7·48	—	4·16	—	13·70
IV[f,h]	2·33[d]	—	6·33[d]	7·53	—	3·92	—	14·03
IV[g]	1·56	4·72	3·02	6·96	6·98	3·86	9·80	13·88
V	2·37[d]	—	6·39[d]	7·53	—	4·19	—	13·72

[a] Doublet, $J = 6{\cdot}1$ Hz; [b] multiplet; [c] apparent doublet, an AB part of an ABX system; [d] singlet; [e] exchangeable singlet; [f] quinone part; [g] phenolic part; [h] dehydro-part.

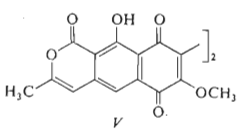
xanthomegnin (*III*). The agreement of both the UV/VIS and ^{1}H-NMR spectra with published data[6] is satisfactory. Its ^{13}C-NMR spectrum (Table II) contains, besides fifteen signals originating from the quinoid xanthomegnin part, the signals assignable to the dehydro-part of the molecule. All observed data are compatible with structure *III*.



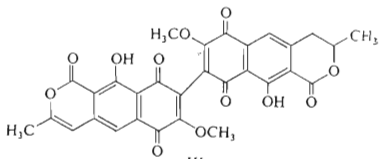
I



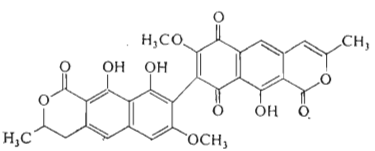
II



V



III



IV

The ^{1}H-NMR spectrum of the minor metabolite *IV* (Table I) is similar to that of viomellein. However, it also contains the signals of the protons from the dehydro-part of molecule *III*. Its mass spectrum exhibits a peak m/z 560 ($C_{30}H_{24}O_{11}$), *i.e.*, two mass units smaller than viomellein. Also this ion is a M+2 ion caused by protonation of the quinone system[5]. Thus, the structure of 3',4'-dehydroviomellein (*IV*) has been assigned to this compound.

The less abundant metabolite exhibits an ion m/z 574 ($C_{30}H_{22}O_{12}$) in its mass spectrum. However, its ^{1}H-NMR spectrum can account for nine protons only. Chemical shifts and multiplicities of these five observed signals fit neatly that of the dehydro-part of *III* (ref.[6]) and hence the actual summary formula is $C_{30}H_{18}O_{12}$ and the m/z 574 ion is a M+4 peak due to the protonation of two quinone systems. Consequently, the compound under discussion is a bisdehydro derivate of xanthomegnin and has the structure of *V*.

When the mycelium was extracted with ethanol, a complex mixture was obtained. The main components of this mixture were identified as 7-ethoxy- and 7,7'-diethoxy analogues of xanthomegnin (^{1}H- and ^{13}C-NMR). The formation of these artifacts can be explained by reesterification, during which methoxyquinones react as vinylogs of methyl esters of carboxylic acids[10]. Their formation can be prevented by suitable choice of solvents.

The main component (*I*) of the produced pigment mixture was already found to be present in the imperfect stage of fungi belonging to the genera *Penicillium*[6,11], *Aspergillus*[12], *Trichophyton*[13–15] and above mentioned *M. cookei*[4] which is the imperfect stage of *N. cajetani*. It is probable that some closely unspecified pigments found in *M. cookei* such as aurosporin, violosporin, citrosporin and rubrosporin[4] are identical to *II – V*, respectively, as identified by us in *N. cajetani*. Compound *II* has been previously described in two species belonging to the genera *Penicillium*[6,11] and *Aspergillus*[12]. The presence of *III* has been demonstrated in *Penicillium citereo-*

TABLE II
^{13}C-NMR Parameters of Compounds *I—III*

Atom	*I*		*J*[c]	*II*[d]	*II*[e]	*III*[e]	*III*[f]
	δ[a]	mult.[b]		δ	δ	δ	δ
1	162·3	d	4·0	171·4	162·6	162·1	164·3
3	74·5	D mt	158·3	76·5	74·1	74·2	145·3
4	36·1	T mt	130·4	34·6	36·4	35·9	104·0
4a	148·3	t	3·9	134·1	148·4	148·0	145·3
5	117·0	D t	169·0, 2·9	116·2	116·6	116·6	115·0
5a	123·1	d	2·9	140·6	123·7	123·5	123·5
6	180·0	d	3·9	97·9	180·4	179·7	179·7
7	158·1	q	3·9	160·4	158·4	160·1	160·1
8	134·8	s		105·1	134·5	134·6	135·3
9	186·2	s		161·7	188·5	158·8	185·8
9a	114·7	dd	7·8, 3·9	99·9	114·9	114·6	114·6
10	162·8	s		155·4	163·0	162·6	162·6
10a	117·7	mt		108·0	117·7	117·5	117·5
3-CH_3	20·5	Q	127·9	20·6	20·7	20·3	19·6
7-OCH_3	61·5	Q	148·4	56·0	60·3	61·2	61·2
S[g]		0·9776			0·9729		

[a] Chemical shifts expressed in the δ-scale; [b] multiplicity in the proton-coupled spectrum, capital letter stands for direct coupling, lower case one for the geminal and vicinal couplings; s singlet, d doublet, t triplet, q quartet, mt multiplet; [c] magnitude of the coupling in Hz; [d] phenolic part; [e] quinone part; [f] dehydro-part; [g] similarity index[7] calculated using the data taken from[6].

viride[6] only. Metabolites *IV* and *V* have not yet been found in nature. This survey indicates that the biosynthesis of metabolites belonging to the xanthomegnin family might be quite widespread among imperfect fungi and evidently also in the *Ascomycetes*.

The inhibition effect of *I* and *II* upon growth of gram-positive and gram-negative bacteria was described recently[6]. Their effect can be related to an earlier finding[16] that the compound *I* uncouples oxidative phosphorylation in the rate liver mitochondria. Distinct inhibition zones using the plate agar diffusion test with *Bacillus subtilis* were observed for these pigments at the following concentration levels: 10 (*II*, *IV*), 50 (*I*), and 100 μg/ml of an ethanolic solution (*III*). The larger inhibition effect of the partially reduced dimeric quinones *II* and *IV* is probably due to their better solubility. The eventual use of these compounds as antibiotics is highly improbable since they are distinct mycotoxins[17].

EXPERIMENTAL

Melting points were determined in the Kofler hot-stage apparatus. UV/VIS spectra were measured in methanol and 0·1M methanolic NaOH on a Cary 118 spectrometer. IR spectra were measured in KBr pellets on a Perkin–Elmer spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded using a Jeol FX-60 instrument (59·797 and 15·036 MHz, FT mode) in $CDCl_3$ with tetramethylsilane as an internal standard at 25°C. Chemical shifts were calculated with accuracy ±0·005 and ± 0·06 ppm from the digitally obtained address differences. Mass spectra were measured on a Varian MAT-311 mass spectrometer at 11 aJ (70 eV), ion source temperature 300°C, direct inlet temperature 250—270°C. The error of high resolution measurements was ±5 ppm. Samples were dried before analysis several hours in high vacuum. Thin-layer chromatography (TLC): Silufol-20 plates (Kavalier Glassworks, Votice, Czechoslovakia) with a thick sorbent layer pretreated by rinsing in 0·25M oxalic acid in methanol, dried and activated for 10 minutes at 105°C; solvent system: chloroform–aceton 20 : 1 (S1), saturated with oxalic acid. The sensitivity of detection of the components was enhanced by spraying with 2M-NaOH; the colours of all antibiotic spots turned to violet. Column chromatography: silica gel (Kieselgel 50—100 μ, Serva Feinbiochemica, Heidelberg, FRG) impregnated with 0·25M oxalic acid; system S1; column 75× 6 cm, flow rate 350 ml/h, sample loading 5·6 g. Antibiotic activity was determined by the agar diffusion test using *Bacillus subtilis* as a test organism. Application: holes 8 mm diameter, 0·1 ml of ethanolic solution of *I*—*IV* at concentrations 10, 50 and 100 μg/ml.

Production and Isolation of Metabolites

Strain *N. cajetani* UAMH 3325 (University of Alberta Mold Herbarium, Edmonton, Canada) was stored on solid malt medium (Difco) at 25°C. The 0·5 l flasks (reciprocal shaker, 2 Hz) containing 80 ml of 8% wort medium were inoculated using agar cultures of the mold. After six days of growth at 22°C the cultures were transferred into 2 l flasks containing 350 ml of the same medium, which were used for the inoculation of the fermentors. The mycelium used for extraction was cultivated 12 days in two laboratory fermentors at 24°C, aeration of 8 l air/min, stirring 6 Hz. Each fermentor contained 10 l of 8% wort medium and 50 ml of antifoaming agent (polypropylene glycol 2025, BDH Chemicals, Poole, England).

The grown mycelium was filtered off (using cheese cloth), washed with water and lyophilized. Pulvered lyophilizate (226 g) was extracted with chloroform (7 × 2 l) at room temperature. The resulting brown-red extract was concentrated on a rotary vacuum evaporator and the residue (18 g) was solidified after light petroleum extraction. Re-extraction of this solid with chloroform followed by solvent removal yielded a dark brown crude powder of the pigment fraction (5·6 g). Three brownish-red diffuse zones were eluted during column chromatography on silica gel impregnated with oxalic acid (volumes 600, 500 and 800 ml, fractions A, B and C). Other zones of different color were observed towards the top of the column, but were not eluted. Antibiotic pigments contained in fractions A—C were further purified by repeated thinlayer chromatography. Colored zones on the developed sheets were cut out and pure compounds extracted with chloroform.

Xanthomegnin (I): main component of fraction C (R_F 0·26), obtained by crystallization from a concentrated chloroform solution as orange plates (890 mg) which decomposed without melting above 265°C and exhibited inverse temperature dependence of solubility. UV/VIS spectrum (methanol): λ_{max} 398, 275 nm; (methanol–NaOH): 540, 398, 265 nm. IR spectrum: 3415, 1720, 1680, 1620, 1595, 1555 cm^{-1}. ^{1}H- and ^{13}-NMR spectrum: see Tables I and II. Mass spectrum: *m/z* 578 (40, $C_{30}H_{26}O_{12}$, M+4), 576 (25, $C_{30}H_{24}O_{12}$, M+2), 574 (13, $C_{30}H_{22}O_{12}$, M), 560 (40, $C_{30}H_{24}O_{11}$), 544 (72, $C_{29}H_{20}O_{11}$), 529 (87, $C_{28}H_{17}O_{11}$), 527 (100, $C_{28}H_{15}O_{11}$), 511 (95, $C_{28}H_{15}O_{10}$), 500 (37, $C_{28}H_{20}O_9$), 485 (65, $C_{27}H_{17}O_9$), 483 (47, $C_{27}H_{15}O_9$), 187 (25, $C_{11}H_7O_3 + C_{15}H_7$, 1 : 1), 195 (35, $C_{10}H_7O_2$).

Viomellein (II): main pigment of the fraction A (R_F 0·45), obtained by crystallization from the mixture light petroleum–chloroform; red microcrystals (105 mg), decomposing above 260°C with discoloration. UV/VIS spectrum (methanol): λ_{max} 376, 366 (sh), 264 nm; (methanol–NaOH): 536, 385, 372 (sh), 330, 265 nm. IR spectrum: 3445, 1735, 1675, 1640, 1587 (sh) cm^{-1}. ^{1}H- and ^{13}C-NMR spectrum: see Tables I and II. Mass spectrum: *m/z* 562 (100, $C_{30}H_{26}O_{11}$, M+2), 544 (21, $C_{30}H_{24}O_{10}$), 529 (93, $C_{29}H_{21}O_{10}$), 511 (50, $C_{29}H_{19}O_9$), 484 (81, $C_{28}H_{20}O_8$).

3,4-*Dehydroxanthomegnin* (III): compound with R_F 0·34 present in fraction B, obtained as a red powder (45 mg) by precipitation from a chloroform solution using tetrachloromethane, decomposing without melting above 160°C. UV/VIS spectrum (methanol): λ_{max} 436 (sh), 385, 275 (sh), 237 nm; (methanol–NaOH): 550, 394, 300 (sh), 275 (sh), 254 (sh) nm. IR spectrum: 3385, 1738, 1680, 1655, 1618, 1590 (sh), 1548 (sh) cm^{-1}. ^{1}H- and ^{13}C-NMR spectrum see Tables I and II. Mass spectrum *m/z*: 576 (14, $C_{30}H_{24}O_{12}$, M+4), 574 (15, $C_{30}H_{22}O_{12}$, M+2), 572 (10, $C_{30}H_{20}O_{12}$, M), 558 (24, $C_{30}H_{22}O_{11}$), 542 (48, $C_{29}H_{18}O_{11}$),527 (100, $C_{28}H_{15}O_{11}$), 483 (45, $C_{27}H_{15}O_9$).

3′,4′-*Dehydroviomellein* (IV): minor component from fraction A (R_F 0·52), obtained by precipitation from a concentrated chloroform solution using tetrachloromethane; a purple powder (16 mg) decomposing without melting above 150°C. UV/VIS spectrum (methanol): λ_{max} 446, 375, 365 (sh), 261 nm; (methanol–NaOH): 547, 383, 372 (sh), 260, 230 (sh) nm. IR spectrum: 3390, 1752, 1675 (sh), 1648, 1618, 1595 cm^{-1}. ^{1}H-NMR spectrum: see Table I. Mass spectrum: *m/z* 560 (100, $C_{30}H_{24}O_{11}$, M+2), 546 (41, $C_{30}H_{26}O_{10}$), 542 (22, $C_{30}H_{22}O_{10}$), 528 (85, $C_{29}H_{20}O_{10}$), 527 (80, $C_{29}H_{19}O_{10}$), 512 (42, $C_{29}H_{20}O_9$).

3,4,3′,4′-*Bisdehydroxanthomegnin* (V): minor component from fraction A (R_F 0·43), isolated during TLC re-chromatography of *II*: a dark red amorphous powder (2 mg). UV/VIS spectrum (methanol): λ_{max} 364, 267 (sh), 231 (sh) nm; (methanol–NaOH): 530, 387, 282, 229 nm. ^{1}H-NMR spectrum is given in Table I. Mass spectrum: *m/z* 574 (100, $C_{30}H_{22}O_{12}$, M+4), 542 (67, $C_{29}H_{18}O_{11}$).

Authors are indebted to Dr J. W. Carmichael, University of Alberta, Edmonton, Canada, for providing the strain UAMH 3325 of Nannizzia cajetani *used throughout this investigation. Dr J. Weijer akcnowledges with gratitude his indebtedness to the Czechoslovak Academy of Sciences and to the Natural Sciences and Engineering Research Council Canada for their generous support.*

REFERENCES

1. Ajello L.: Sabouraudia *1*, 173 (1961).
2. Ajello L.: Mycologia *58*, 970 (1966).
3. Ajello L.: Mycologia *51*, 69 (1959).
4. Nozawa Y., Ito Y.: Experientia *26*, 803 (1970).
5. Zellner K. P. in the book: *The Chemistry of the Quinoid Compounds* (S. Patai, Ed.), p. 231. Wiley, London 1974.
6. Zeeck A., Russ P., Laatsch H., Loeffler W., Wehrle H., Zähner H., Holst H.: Chem. Ber. *112*, 957 (1979).
7. Bremser W., Klier M., Meyer E.: Org. Magn. Resonance *7*, 97 (1975).
8. Höfle G., Röser K.: J. Chem. Soc., Chem. Commun. *1978*, 611.
9. Takeuchi S., Uzawa J., Seto H., Yonehara H.: Tetrahedron Lett. *1977*, 2943.
10. Ulrich H., Richter R. in the book: *Methoden der Organischen Chemie* (Houben-Weyl), Band VII/3a (C. Grundmann, Ed.), p. 18. Thieme, Stuttgart 1977.
11. Stack M. E., Eppley R. M., Dreifuss P. A., Pohland A. E.: Appl. Environ. Microbiol. *33*, 351 (1977).
12. Durley R. C., MacMillan J., Simpson T. J., Glen A. T., Turner W. B.: J. Chem. Soc., Perkin Trans. *1*, 1975, 163.
13. Wirth J. G., Beesley T. E., Anand S. R.: Phytochemistry *4*, 505 (1965).
14. Blank F., Day W. C., Just G.: J. Invest. Dermatol. *40*, 133 (1963).
15. Ng A. S., Just G., Blank F.: Can. J. Chem. *47*, 1223 (1969).
16. Kawai K., Akita T., Nishibe S., Nozawa Y., Ogihara U., Ito Y.: J. Biochem. *79*, 145 (1976).
17. Carlton W. W., Stack M. E., Eppley R. M.: Toxicol. Appl. Pharmacol. *38*, 455 (1976).

Translated by the author (P. S.).