UDC 547.92: 582.237

119. Shoji Shibata, Shinsaku Natori, Ko Fujita, Isao Kitagawa,* and Kazue Watanabe**: Metabolic Products of Fungi. XV.*** Pachymic Acid, a Constituent of "Bukuryo" (Fu Ling 茯苓), a Sclerotium of *Poria cocos* (Schw.) Wolf.

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A sclerotium of a basidiomycete fungus, *Poria cocos* (Schw.) Wolf (syn. *Pachyma Hoelen* Rumph.), specially found on pine tree roots, is most commonly used as a principal medicament in Chinese medicine and named Bukuryo (Fu Ling 茯苓), on which some chemical studies have been reported.[1~10] A polysaccharide component, pachymose or pachyman,[1,3] has recently been reported to be a polyglucose with 1-3 β-linkage.[7] Nakanishi, *et al.*[8] isolated an acidic triterpenoid compound from the crude drug and designated it pachymic acid. It was proposed that pachymic acid, m.p. 300°, possesses a molecular formula $C_{30}H_{44}O_5$, containing one each of hydroxyl, carboxyl, and lactone groups with one unsaturated bond.

Recently, Robertson and his co-workers[9] examined the mycelial products of artificially cultivated *Poria cocos* and found that, depending on the strains, eburicoic acid or tumulosic acid was produced accompanying the respective dehydro derivatives.

Iseda and Yagishita[10] obtained a mixture of eburicoic and dehydroeburicoic acids from naturally occurring sclerotia of *Poria cocos*. Thus the presence of pachymic acid has never been proved by the foregoing workers.

Presence of pachymic acid in the sclerotium of *Poria cocos* was re-examined and an acid, m.p. 296~298°(corr.), was isolated from the sparingly soluble fraction of the ethereal extract.

The acid should be pachymic acid, since it gave an acetate, methyl ester, and acetate of methyl ester, whose melting points were nearly the same as those recorded by Nakanishi, *et al.*[8] However, the molecular formula of pachymic acid should be altered to $C_{33}H_{52}O_5$, with which the present analyses agreed well.

Nakanishi, *et al.* considered that pachymic acid is a lactonic acid which could be recovered unchanged on boiling with alkali followed by acidification, but their evidence was doubtful, and contrary to their recognition, the present result showed that the alkali-treated product afforded a methyl ester, m.p. 165~166°, which was proved to be evidently different from the methyl ester of pachymic acid, m.p. 185~187°, whereas the acetate of the above product was shown to be identical with O-acetylpachymic acid.

Hence it appeared that pachymic acid is an acetate which is readily deacetylated with alkali. Nakanishi, *et al.* must have taken the deacetylated pachymic acid for the original

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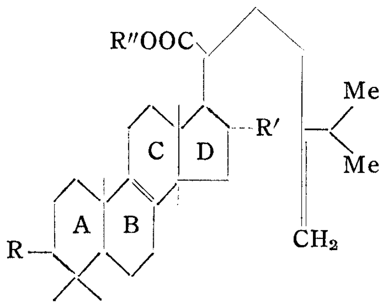
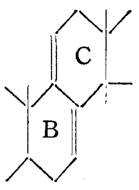
pachymic acid, since the melting points of the two compounds could not so clearly be distinguished.

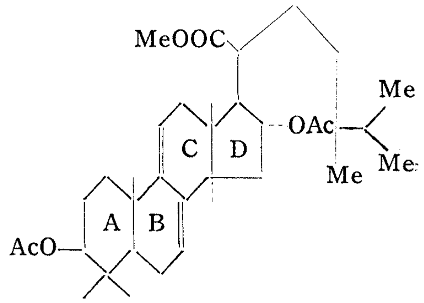
The infrared spectrum of pachymic acid showed a strong characteristic band at 1256 cm^{-1} which was assigned to acetyl C–O absorption, and was absent from the spectrum of methyl ester of the alkali-treated product. It was therefore assumed that pachymic acid is O-acetylpolyporenic acid-B[11] by which all the properties and behavior of pachymic acid and its derivatives so far prepared were explicable. The ultraviolet spectrum of pachymic acid showed the contamination of its corresponding dehydro derivative in about 10%, which was practically inseparable.

Methyl O-acetyldihydrodehydropachymate was prepared from pachymic acid for identification to avoid any possible uncertainty which might occur due to the contamination of dehydro derivative in the original acid.

By the courtesy of Professor E. R. H. Jones and Dr. T. G. Halsall of the Oxford University, who sent us the samples of "polyporenic acid-B" derivatives, we established the respective identities of "methyl polyporenate-B" (V) and its 3β-acetate (IV) with methyl deacetylpachymate and methyl pachymate by mixed fusion and comparison of infrared spectra. Pachymic acid was, therefore, established to be 3β-O-acetylpolyporenic acid-B (I). The components contained in the ether-soluble portion are now under investigation and it seems quite certain that eburicoic acid would be present.







(I) R=OAc, R′=OH, R″=H "Pachymic acid"
(II) R=R′=OH, R″=H "Polyporenic acid-B"
(III) R=R′=OAc, R″=H
(IV) R=OAc, R′=OH, R″=Me
(V) R=R′=OH, R″=Me
(VI) R=R′=OAc, R″=Me
(VII) R=R′=OAc, R″=Me, Me in place of $>CH_2(28)$

(VIII)

Robertson and his co-workers[13] found that O-acetyleburicoic acid was isolated from the mycelium of *Polyporus anthracophilus* Cooke grown naturally on the wood of *Eucalyptus regnans* while free eburicoic acid was produced from the artificially cultivated fungus.

Regarding this, it would be quite possible, as observed by Robertson and his co-workers,[9] that artificially cultivated mycelium of *Poria cocos* can only produce deacetylpachymic acid, viz. tumulosic and dehydrotumulosic acids.

The authors are grateful to Professor E.R.H. Jones and Dr. T.G. Halsall of the Oxford University for making available the samples of "polyporenic acid-B" derivatives. They also thank Drs. K. Nakanishi and S. Yamamura, University of Nagoya, for measuring optical rotations, Mr. K. Tanikawa of this Faculty for infrared spectral determination, and Miss H. Yamanouchi, Institute for

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[11] The name "polyporenic acid-B" proposed by Jones and his co-workers[12] is used to represent a natural mixed acid which consists of tumulosic and dehydrotumulosic acids.[9] The name of "pachymic acid" here used represents the 3β-acetate of mixed acid "polyporenic acid-B".

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Applied Microbiology, Mrs. S. Nakagome, Institute for Infectious Diseases, University of Tokyo, for elementary analyses. This work was supported partly by a Grant in Aid for Scientific Research from the Ministry of Education, to which the authors are also grateful.

Experimental

All m.p.s were determined in H_2SO_4 bath and corrected. Ultraviolet spectra were determined with a Unicam spectrophotometer, and infrared spectra with a Hilger H-800, and Koken 301 recording spectrophotometers. Optical rotations were measured by Rudolf photoelectric polarimeter.

Extraction and Isolation of Pachymic Acid—Minced naturally occurring "Bukuryo", sclerotium of *Poria cocos* (SCHW.) WOLF. (1 kg.) was exhaustively extracted with ether. The combined ethereal extract was concentrated to about 500 cc., from which colorless crystals separated out after standing for several days. Yield of the crude crystals was around 0.06%. The ethereal mother liquor contained a mixture of presumably related compounds (yield, 0.6%).

Pachymic Acid (3β-O-Acetylpolyporenic Acid-B) (I)—Repeated crystallization of the crude product from MeOH gave colorless needles of m.p. 296~299°; $[\alpha]_D^{22.5}$ +17.7° (pyridine, c=0.566). *Anal.* Calcd. for $C_{33}H_{52}O_5$: C, 74.96; H, 9.91. Found: C, 74.85, 74.78; H, 10.16, 9.58. U.V. λ_{max}^{EtOH} mμ (log ε): 235 (3.08), 242 (3.12), 251 (2.95). I.R. ν_{max}^{Nujol} cm^{-1}: 1738 (ester C=O), 1705 (COOH C=O), 1645 (C=C), 1256 (acetyl C-O); 895 (δ >C=CH_2).

O-Acetylpachymic Acid (3β, 16α-Di-O-acetylpolyporenic Acid-B) (III)—Obtained by acetylation of pachymic acid (I) or deacetylpachymic acid (II) as colorless needles (from EtOH), m.p. 218~221°; $[\alpha]_D^{22.1}$ +10.0° (pyridine, c=0.519). *Anal.* Calcd. for $C_{35}H_{54}O_6$: C, 73.64; H, 9.54. Found: C, 73.59, 73.72; H, 9.37, 9.41.

Methyl Pachymate (Methyl 3β-O-Acetylpolyporenate-B) (IV)—Pachymic acid was methylated with CH_2N_2 in ether solution and the product was chromatographed on Al_2O_3 inactivated with AcOH and MeOH, using benzene as a developing solvent. A fraction which gave colorless crystals was recrystallized from MeOH to form needles, m.p. 185~187°; $[\alpha]_D^{11.5}$+41.5° ($CHCl_3$, c=0.572). *Anal.* Calcd. for $C_{34}H_{54}O_5$: C, 75.23; H, 10.03. Found: C, 75.13, 75.27; H, 9.81, 9.75. U.V.: λ_{max}^{EtOH} mμ (log ε) 233 (3.25), 242 (3.28), 251 (3.11). I.R. $\nu_{max}^{CCl_4}$ cm^{-1}: 1732 (ester C=O), 1647 (C=C), 1248 (acetyl C-O) 897 (δ >C=CH_2).

Admixture with methyl 3β-O-acetylpolyporenate-B[11] showed no depression of the melting point and the infrared spectra of the two samples were superimposable.

Methyl O-Acetylpachymate (Methyl 3β, 16α-Di-O-acetylpolyporenate-B) (VI)—Acetylation of methyl pachymate (IV) and of methyl deacetylpachymate (V) or methylation of O-acetylpachymic acid (III) gave the same colorless needles which were recrystallized from MeOH. m.p. 157.5~159.5°; $[\alpha]_D^{14.5}$ +5.3° ($CHCl_3$, c=0.450). *Anal.* Calcd. for $C_{36}H_{56}O_6$: C, 73.93; H, 9.65. Found: C, 73.90; H, 9.39.

Deacetylpachymic Acid (Polyporenic Acid-B) (II)—On boiling with EtOH-KOH for 10 hrs., pachymic acid (I) or methyl pachymate (IV) afforded deacetylated product, which formed a gelatinous mass and turned into crystalline powder on treatment with ethyl acetate. m.p. 308~310°; $[\alpha]_D^{22.1}$ +10.7° (pyridine, c=0.878).

Methyl Deacetylpachymate (Methyl Polyporenate-B) (V)—Deacetylpachymic acid (II) was methylated with CH_2N_2 in Et_2O solution, the product was chromatographed on neutralized Al_2O_3 column, and finally recrystallized from MeOH to give colorless needles, m.p. 165~166°; $[\alpha]_D^{11.5}$ +23.6° ($CHCl_3$, c=0.414).

On treatment of methyl O-acetylpachymate (VI) or methyl pachymate (IV) with 5% MeOH-KOH, allowing to stand at 27° for 20 hrs., the above product was obtained. *Anal.* Calcd. for $C_{32}H_{52}O_4$: C, 76.75; H, 10.47. Found: C, 76.93; H, 10.60. I.R. $\nu_{max}^{CCl_4}$ cm^{-1}: 1737 (ester C=O), 1645 (C=C); 894 (δ >C=CH_2).

The identity of methyl deacetylpachymate and methyl polyporenate-B was confirmed by a mixed fusion and comparison of the infrared spectra.

Methyl O-Acetyldihydropachymate (Methyl 3β, 16α-Di-O-acetyldihydropolyporenate-B) (VII)—Methyl O-acetylpachymate (VI) was hydrogenated in the presence of Pt catalyst. Recrystallization from MeOH gave colorless needles, m.p. 181~182.5°; $[\alpha]_D$ +5.7° ($CHCl_3$, c=0.334). *Anal.* Calcd. for $C_{36}H_{58}O_6$: C, 73.68; H, 9.96. Found: C, 73.84; H, 9.88. U.V. λ_{max}^{EtOH} mμ (log ε): 235 (3.14), 242 (3.20), 251 (3.00).

Methyl Di-O-acetyldihydrodehydrotumulosate[9] (Methyl 3β,16α-Diacetoxyeburico-7, 9(11)-dien-21-oate) (VIII)—A mixture of methyl O-acetyldihydropachymate (VII) (0.10 g.) and SeO_2 (0.23 g.) was boiled in AcOH (5 cc.) and H_2O (0.2 cc) for 8 hrs. The product was chromatographed on Al_2O_3 and recrystallized from MeOH to give colorless needles, m.p. 174~174.5°; $[\alpha]_D^{10.5}$+25.1° ($CHCl_3$, c=0.519).

Anal. Calcd. for $C_{36}H_{56}O_6$: C, 73.93; H, 9.65. Found: C, 73.85; H, 9.65. U.V. $\lambda_{\max}^{\mathrm{EtOH}}$ mμ (log ε): 235 (4.14), 242 (4.20), 251 (4.00). I.R. $\lambda_{\max}^{\mathrm{Nujol}}$ cm^{-1}: 1724 (ester C=O), 1252 (acetyl C-O); 813 (δ—CH=C<).

Summary

Pachymic acid which was isolated by Nakanishi, *et al.*[8] from a Chinese drug "Bukuryo (Fu Ling)" (a sclerotium of *Poria cocos* (SCHW.) WOLF) has been proved to be 3β-O-acetylpolyporenic acid-B.

(Received May 31, 1958)

UDC 547.823.07

120. Torizo Takahashi und Fumiro Yoneda: Über die Synthese der heterozyklischen Verbindungen mit Stickstoff. CXIV.[1)] Die Merkurierung der Oxypyridine.

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Was die Merkurierungen der Pyridinabkömmlinge anbetrifft, wurden die von Pyridin,[2] 4-Oxypyridin-N-oxyd,[3] 2-Pycolin[4] sowie 2-Aminopyridin[5] bereits beschrieben. Diesen Angaben nach wird das Wasserstoffatom des Pyridinrings ziemlich leicht durch das Quecksilber substituiert, beim 2-Pycolin aber erleidet ausnahmsweise seine Seitenkette, die Methylgruppe, eine Merkurierung.

In vorliegender Mitteilung berichten wir über die Erkenntnisse, die wir beim Merkurieren der Oxypyridine gewonnen haben.

Erstens liess sich 2-Oxy-3,5-pyridin-bis(merkuriacetat) (I) dadurch in guter Ausbeute erhalten, dass man Quecksilberacetat auf das 2-Oxypyridin in der Wasserlösung einwirken liess. In diesem Falle scheidet die Reaktionslösung bei Zimmertemperatur wenige farblose Nadeln (wahrscheinlich als Additionsprodukte anzusehen) aus. Diese Kristalle verschwinden einmal beim Erwärmen auf dem Wasserbade; durch weitere Erwärmung scheidet sich dann (I) ab. Während wir diese Merkurierung unter verschiedenen Bedingungen versuchten, gelang es uns nicht, ein einfach merkuriertes Produkt zu erhalten. Diesbezüglich haben Binz und Maier-Bode[6] 2-Oxypyridin-5-merkurichlorid durch Überführen aus 2-Oxypyridin-5-arsinsäure hergestellt.

(I) liefert nun beim Behandeln mit gesättigter Kochsalzlösung 2-Oxy-3,5-pyridin-bis-(merkurichlorid) (II). Letzteres wurde durch die Einwirkung einer Jod-Jodkalium-Lösung in 2-Oxy-3,5-dijodpyridin (III) übergeführt. Dieses Substanz schmilzt ebenfalls in Übereinstimmung mit den bekannten Angaben[7,8] bei 265°.

Die Merkurierung des 4-Oxypyridins verläuft ungefähr unter gleichen Bedingungen wie beim 2-Oxypyridin: Es ergibt nämlich 4-Oxypyridin durch Einwirkenlassen von Queck-

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