found to be a natural product[5]. As an intermediate of the synthesis by MACPHILLAMY and ELDERFIELD[4], the methylglycoside diacetate (III or IV or a mixture of both) was prepared also; unfortunately this derivative was not purified and characterized. We repeated the synthesis, starting from authentic L-rhamnose, and isolated the methyl glycoside diacetate by chromatography on silica gel. The semi-solid product showed a single spot on thin-layer chromatography but the NMR-spectrum showed the presence of 2 anomeric glycosides in a ratio of about 3:1, the predominant component being different from the degradation product, mp 70–71°, of aranciamycin. By crystallization, a single compound of mp 112–113° and $[\alpha]_D = +77°$ (in methanol) was obtained. This proved later to be the $\beta$-glycoside VI (see below).

Table I. NMR-spectrum of the mixture of $\alpha$- and $\beta$-1,3,4-tri-*O*-acetyl-2-*O*-methylrhamnose (4:1), $CDCl_3$, 100 MHz

| $\delta$ | Splitting | J | Integral | Assignments |
|---|---|---|---|---|
| 1.18 | d | 6 Hz | ca. 2.4 H | $CH_3$ of $\alpha$-anomer |
| 1.22 | d | 6 | ca. 0.6 H | $CH_3$ of $\beta$-anomer |
| 2.00 | s | | 3 H | 3 OAc (both anomers) |
| 2.04 | s | | 3 H | |
| 2.10 | s | | 3 H | |
| 3.45 | s | | ca. 2.4 H | $OCH_3$ ($\alpha$) |
| 3.54 | s | | ca. 0.6 H | $OCH_3$ ($\beta$) |
| 3.58 | t | 1.5 | ca. 0.8 H | H–C–2 ($\alpha$) |
| ca. 3.85 | m | | ca. 1 H | H–C–5 (both anomers) |
| ca. 5.1 | complex | | 2 H | H–C–3, H–C–4 (both an.) |
| 5.69 | s | | ca. 0.2 H | H–C–1 ($\beta$) |
| 6.08 | d | 1.5 | ca. 0.8 H | H–C–1 ($\alpha$) |

Table II. NMR-spectra of the anomeric methylglycoside diacetates of 2-*O*-methyl-L-rhmnose, $CDCl_3$, 100 MHz

| $\beta$-Anomer (mp 112°) | | | $\alpha$-Anomer (mp 70°) | | | |
|---|---|---|---|---|---|---|
| $\delta$ | Splitting | Integral | $\delta$ | Splitting | Integral | Assignments |
| 1.24 | d, 6 Hz | 3 H | 1.20 | d, 6 Hz | 3 H | $CH_3$–6 |
| 2.00 | s | 3 H | 2.02 | s | 3 H | OAc |
| 2.05 | s | 3 H | 2.05 | s | 3 H | OAc |
| 3.53 | s | 3 H | 3.40 | s | 3 H | $OCH_3$ |
| 3.56 | s | 3 H | 3.48 | s | 3 H | $OCH_3$ |
| 3.2–3.6 | m | 1 H | ca. 3.8 | m | 1 H | H–C–5 |
| 3.70 | d, 2.5 Hz | 1 H | 3.64 | t, 1.5 Hz | 1 H | H–C–2 |
| 4.41 | s | 1 H | 4.73 | d, 1.5 Hz | 1 H | H–C–1 |
| 4.7–5.3 | complex | 2 H | 510–5.3 | complex | 2 H | H–C–3 and H–C–4 |

The mother liquors, a mixture of both anomers, were epimerized (*p*-toluenesulfonic acid in methanol, 4 h, reflux) and reacetylated to an oily product which was the almost pure $\alpha$-glycoside diacetate III. After chromatographic purification and crystallization from petroleum ether, colorless prisms of mp 69° were obtained whose Rf value, NMR- and IR-spectra were identical with those of the degradation product of aranciamycin[2]. Since the synthetic as well as the natural derivative show $[\alpha]_D = -69°$ (methanol), the sugar component of aranciamycin is 2-*O*-methyl-L-rhamnose (I).

The assignments of the anomeric configurations of the two crystalline methylglycoside diacetates again follow from SINCLAIR's and SLEETER's rule[3] (see above). The derivative of mp 112° shows the $CH_3$ doublet at lower field (Table II) and is therefore the $\beta$-glycoside VI while the anomer of mp 70° is the $\alpha$-glycoside III. This assignment is confirmed by the optical rotations. According to HUDSON's rule[6], the L-$\alpha$-glycoside (mp 70°) is levorotatory and the L-$\beta$-glycoside (mp 112°) is dextrorotatory.

In aranciamycin itself the signal of H–C–1 of the sugar moiety occurs at $\delta$ 5.49 ppm (KELLER-SCHIERLEIN et al.[2], Figure 3) as a singlet. This corresponds to the singlet signals of H–C–1 in the $\beta$-triacetate V (Table I) and the $\beta$-methylglycoside diacetate VI (Table II). Aranciamycin is therefore a $\beta$-2-*O*-methyl-L-rhamnoside of aranciamycinone. Since aranciamycin shows 2 secondary (acetylable) alcoholic hydroxyl groups, while aranciamycinone contains 1 such group[2], the secondary hydroxyl of the aglycone is involved in the glycosidic linkage of the antibiotic. The partial structure of the aglycone[2] given earlier can be extended to the partial formula VII for aranciamycin[7].

*Zusammenfassung.* Der Zuckerbaustein des Antibiotikums Aranciamycin ist die 2-*O*-Methyl-L-rhamnose.

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## Heartwood Constituents of *Pinus formosana*

The formosan pine, *Pinus formosana* Hayata, grows in the mountains of Taiwan and morphologically is considered a variety of the Japanese white pine, *P. parviflora* Sieb. and Zucc. This species has been placed in the group Strobi, subgenus Haploxylon[1]. It will be recalled that the Haploxylon pines contain both flavones and flavanones, as well as stilbene derivatives, while the Diploxylon pines possess only stilbenes and flavones. Generally, pines belonging to these two subgenera can be distinguished by their specific content of heartwood phenolics[2–5]. Since any deviations from a general pattern are of interest in phytochemical work, it was decided to make a brief investigation of this tree.

A benzene extract of the heartwood was treated with sodium hydroxide solution. The aqueous layer was removed, then acidified to precipitate a semi-crystalline oil. Chromatography over silicic acid gave dehydroabietic and isopimaric acids, plus pinostrobin and tectochrysin.

Identification of these products was made by spectral methods, followed by comparison with authentic samples[6]. Neither of these flavonoids have been reported in *P. parviflora*[7], but pinostrobin was detected in *P. formosana* by a paper chromatography screening procedure[8]. It is concluded that the formosan pine is correctly placed in the subgenus Haploxylon[2].

During this investigation, mass spectra were determined for both pinostrobin and tectochrysin. Due to the paucity of such data in the literature[9–11], there is given here a summation of the significant break-down patterns on these two compounds:

Pinostrobin: *m/e* = 270 ($M^+$), 269 ($M^+$-1), 193 ($M^+$-$C_6H_5$), 166 ($C_8H_6O_4^+$), 138 ($C_7H_6O_3^+$), 123 ($C_6H_3O_3^+$), 104 ($C_8H_8^+$), and 77 ($C_6H_5^+$); Tectochrysin: *m/e* = 268 ($M^+$), 240 ($M^+$-CO), 226 (240-$CH_2$), 166 ($C_7H_4O_2^+$), 138 ($C_7H_6O_2^+$), 102 ($C_8H_6^+$), and 77 ($C_6H_5^+$).

*Zusammenfassung.* Die Untersuchung der sauren Anteile eines Extraktes des Kernholzes von *Pinus formosana* zeigte das Vorhandensein von Pinostrobin und Tectochrysin neben Dehydroabietin- und Isodextroprimarsäure. Diese Befunde erlauben, *P. formosana* der Gruppe Haploxylon zuzuteilen.

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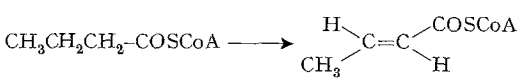
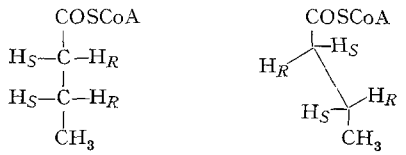
## Zur Stereochemie der enzymatischen Dehydrierung von Butyryl-CoA

Butyryl-CoA-Dehydrogenase (Butyryl-CoA: (Akzeptor) Oxydoreduktase E.C.1.3.2.1), ein FAD enthaltendes mitochondriales Enzym des Fettsäureabbaus, katalysiert die Dehydrierung von Butyryl-CoA zu Crotonyl-CoA[1] (Gl. 1). In vivo ist dieser Oxydationsprozess mit dem Elektronentransportsystem der Atmungskette verbunden; in vitro können auch künstliche Akzeptoren, wie Dichlorophenol-indophenol, Methylenblau oder Kaliumhexacyanoferrat(III) die Elektronen aufnehmen, dies geschieht aber nur dann in ausreichendem Masse, wenn geeignete Mediatoren, wie ein Elektronen transportierendes Protein oder Phenazinmethosulfat, zugegen sind. Im Zusammenhang mit Arbeiten über die mechanistisch ähnliche enzymatische Umwandlung von Bernsteinsäure in Fumarsäure[2] drängte sich eine genaue stereochemische Untersuchung der Reaktion auf. Nachfolgend wird über die Resultate einer solchen Untersuchung berichtet.




(1)




**1**




**2**

Die beiden Methylengruppen des Butyryl-Restes, die in die Reaktion direkt einbezogen werden, sind prochiral[3] und tragen je ein pro-*R*- und ein pro-*S*-Wasserstoffatom (vgl. **1** und **2**). Zur Feststellung der Identität der zur Abspaltung gelangenden H-Atome war es notwendig, an C-2 bzw. C-3 stereospezifisch tritierte Buttersäure-Proben herzustellen. Die eingeschlagenen Synthesewege sind im Schema 1 zusammengefasst.

Enzymatische Reduktion von [1-$^3$H]-Propionaldehyd **3** mit NADH in Gegenwart von Hefe-ADH gab (1*S*)-[1-$^3H_1$]-Propan-1-ol **4**, dessen Tosylat **5** bei der mit Inversion stattfindenden Cyanolyse[4,5] (2*R*)-[2-$^3H_1$]-Butyronitril **6** lieferte. Hydrolyse der Nitrilgruppe von **6** erfolgte über das entsprechende Amid in Anlehnung an eine früher erprobte Methode[4], und führte praktisch ohne Tritiumverlust zu (2*R*)-[2-$^3H_1$]-Buttersäure, **7**. Die dazu enantiomere (2*S*)-[2-$^3H_1$]-Buttersäure **9** stellte man auf ähnlichem Wege ausgehend von (1*R*)-[1-$^3H_1$]-Propyltosylat **8** dar, das seinerseits durch $S_{N2}$-Solvolyse von **5** mit 2*N* NaOH[6] und nochmaliger Tosylierung zugänglich war. Die vorgenommene Zuordnung der Konfiguration für die Säuren **7** und **9** beruht im wesentlichen auf der wohlbegründeten Annahme, dass die enzymatische Reduktion von Propionaldehyd sterisch gleich derjenigen von Acetaldehyd abläuft[7,8]. Dass diese Annahme zutrifft, wurde durch

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