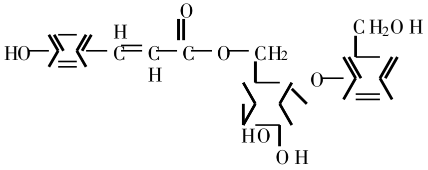
ked at C-6 of glucose, the ^{13}C NMR of glucose′s C-6 should be 68 ppm, but compound Ⅹ is 63.1 ppm. Also, salicin was detected from the alkaline hydrolyzed solution. So we confirmed that salicyl alcohol is linked at C-1 and *p*-hydroxycinnamic acid is linked at C-6 of glucose. Finally, compound Ⅹ was concluded to be β-D-glucopyranoside, 2-(hydroxymethyl)-phenly, 6-[3-(4-hydroxyphenyl)-2-propenoate].



In the literature[4], it was reported that this compound named trichocarposide was isolated from *Populus trichocarpa* C. F. Fang, but its ethylene configuration has not been indicated. In another literature[2] its ethylene bond was defined as cis form. But from our spectra analysis (J= 18 Hz and υ= 120 cm^{-1}) the ethylene bond was confirmed to be trans form.

3.2 *Other compounds*

Besides compounds Ⅲ and Ⅹ, other compounds were elucidated on the basis of chemical evidence and spectra evidence by comparing with authentic samples as β-sitosterol(Ⅰ), stigmasterol(Ⅱ), p-hydroxystyrene(Ⅲ), cinnamic acid (Ⅳ), salicyl alcohol (Ⅴ), pyrocatechol (Ⅵ), protocatechuic acid (Ⅶ), daucosterol(Ⅷ) and salicin (Ⅸ).

References

1 Estes TK, Pearl IA. Studies on the barks of the family Salicaceas Ⅺ. *Tappi*, 1967, **50**(7): 318

2 Pealr IA. Extractives of hardwood wastes as sources of chemicals. *Forest Products Journal*, 1968, **18**(2): 60

抗结核植物药园头柳的化学成分研究

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摘　要　从抗结核植物药中分离并鉴定了 10种成分: β谷甾醇, 豆甾醇, 对羟基苯乙烯, 桂皮酸, 水杨醇, 邻苯二酚, 原儿茶酸, 胡萝卜甙, 水杨甙和毛果柳甙。对羟基苯乙烯是其中抗结核菌作用最强的成分。本文还对文献报道的毛果柳甙的结构进行了修正。

关键词　园头柳; 对羟基苯乙烯; 毛果柳甙

中 国 药 科 大 学 学 报
Journal of China Pharmaceutical University 1996, **27**(5): 271~ 273

Chemical Studies on Anti-tuberculosis Compounds from *Salix Capitata*

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Abstract Ten constituents were isolated from the tender leaves of *S. capitata*. Their structures were identified as: β-sitosterol, stigmasterol, *p*-hydroxystyrene, cinnamic acid, salicyl alcohol, pyrocatechol, protocatechuic acid, ducosterol, salicin and trichocarposide. p-hydroxystyrene shown was effective on inhibiting human mycobasterium tubercolosis. The structure of trichocarposide was corrected according to our expermental data.

Key words *Salix capitata*; *p*-Hydroxystyrene; Trichocarposide

Populus, *Salix* and *Chosenia* genus belongs to Salieaceae, and there are about 540 kinds of these species. In China, there are 200 species of them as genus *Salix*. Some of *Salix species* have anti-inflammatory, analgesic and dedoxicating activities. The leaves of *Salix* species had been used for treating scrofula as Chinese folk medicine. Since 1971, Changli county (52591 hospital) has used *S. capitata* in dosage form the of tablet and injection for treating scrofula shown 93.8% effectiveness on 209 patients. By using this herbal medicine for 20 years, no sideeffect toxicity and tolerance have been produced. Although there are many chemical investigations on genus *Salix*, up to now, we have not seen any report about *S. capitata*. The purpose of this study was to find out the active ingredients from *S. capitata* for treating lymph nodes.

From the EtOAc extracted active portion ten compounds were isolated and their chemical structure were elucidated by means of chemical and spectra methods as: β-sitosterol (Ⅰ), stigmasterol (Ⅱ), *p*-hydroxystyrene(Ⅲ), cinnamic acid (Ⅳ), salicyl alcohol (Ⅴ), pyrocatechol (Ⅵ), protocatechuic acid (Ⅶ), daucosterol (Ⅷ), salicin (Ⅸ) and trichocarposide(Ⅹ). Pharmacological experiments (in vitro) showed that compounds Ⅲ、Ⅳ、Ⅵ、Ⅶ、Ⅷ and Ⅹ inhibited human Mycobactreium tubteculosis H_{37}-RV, but p-hydroxystyrene (Ⅲ) showed more efficiency than the other compounds, MIC= 6.25μg/ml.

Experimental

1 Apparatus

All melting points were determined on XT_4 micro melting point apparatus and were uncorrected. Infra-red spectra were recorded on Shimadzu IR-400 spectrophotometer using KBr discs. NMR spectra were recorded on Bucker AM-300 using TMS as internal standard. EI-MS spectra were taken on JEOL-JMSD-300 instrument at 70 eV.

2 Extraction and isolation

The leaves of *Salix capitata* Y. L. et Skv. were collected from Hebei Province, Changli county. The authentication of species was finished by Prof. Gong Sunlian.

5 kg of the leaves was boiled in water twice, half an hour every time. The water extract was concentrated under reduced pressure and 95% of ethanol was added to

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收稿日期 1996-01-10 [1]本校 1996届坦桑尼亚博士生

make a 70% ethanol solution. The alcohol solution was kept over night and was filtered to remove the insoluble precipitate. The alcohol was removed and the remaining liquid was extracted with EtOAc and n-BuOH. The pharmacological experiment results showed that the EtOAc extract was more effective than other portions.

The EtOAc extract was chromatographied on silica gel column, and eluted with cyclohexane containing increasing amount of ethyl acetate giving compounds Ⅰ-Ⅹ. Compound Ⅲ was purified by sublimation and the others by recrystallization.

3 Structure elucidation

3.1 *Compound* Ⅲ

Compound Ⅲ was purified by sublimation gave colourless needles crystal, mp 72~73℃, and gave a brown colour with FeCl$_3$ reagent. IR υ 3250~3400(-OH), 1675(C=C), 610, 1500(-Ar), 840(para substituted benzene). IR spectrum showed the presence of phenyl hydroxyl and vinyl groups, EI-MS m/z 120(M$^+$, 100), 92(M$^+$-CO, 5.9), 91(51), 65(21). ^{1}H-NMR(DMSO-d$_6$) δ 9.05(1H, s, OH), 7.28(2H, d, J=8.8 Hz), 6.72(2H, d, J=8.8Hz). The above signal consists of two equal AB systems. 6.22(1H, dd, J=17.4 7.7 Hz), 5.55(1H, dd, J=17.4 1.2 Hz), 4.53(1H, dd, J=10.8 1.2 Hz). The above three dd signals are three protons of mono-substituted ethylene. Thus this compound was elucidated as *p*-hydroxystyrene. This compound is very rare to be found in plants due to its easy polymerization and oxidation.

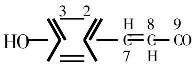
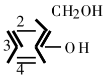
3.2 *Compound* Ⅹ

Colourless plated crystal from dilute ethanol, mp 184.8~185.8℃, gave blue colour with FeCl$_3$ reagent and positive result with Molish reagent. The compound was hydrolyzed with dilute acid and aglycone was identical with authentic sample salicyl alcohol. Compound Ⅹ was also hydrolyzed in alkaline medium and the product was identical with authentic sample *p*-hydroxycinnamic acid. succesive hydrolysis with acid and alkali, only glucose was detected from the mother liquid. So the structure of compound Ⅹ was assumed to be salicyl alcohol and *p*-hydroxycinnamic acid linked up with glucose to produce a double aglycone glucoside. On the basis of hydrolysis condition, it was confirmed that salicyl alcohol is connected with glucose as acetal, while p-hydroxycinnamic acid is connected with glucose as ester.

^{1}H NMR(DMSO-d$_6$), δ 7.56(1H, d, J=18 Hz), 6.39(1H, d, J=18 Hz) This is a pair of trans-ethylene protons of *p*-hydroxycinnamyl. 6.82(2H, d, J=8Hz), 7.54(2H, d, J=8 Hz) are two equal AB system aromatic protons of *p*-hydroxycinamyl. 3.26(2H, s, -CH$_2$OH), 7.33~6.94(4H, m) are four ortho aromatic protons. 5.35(1H) and 4.1~4.8(6H, m) are glucose′s protons.

The ^{13}C NMR spectra data of compound Ⅹ are listed in the following table 1.

Tab 1. ^{13}C NMR chemical shifts of compound Ⅹ (DMSO-d$_6$)

Carbon	Glucose		
1	101.2 d	121.5 s	124.6 s
2	73.3 d	129.6 d	128.8 d
3	76.1 d	115.4 d	114.7 d
4	73.1 d	159.2 s	127.8 d
5	69.9 d	115.4 d	114.7 d
6	63.1 t	129.6 d	131.1 s
7		144.2 d	58.2 t
8		121.5 d	
9		165.7 s	

EI-MS m/z 432(M$^+$, 0.8), 309(M$^+$-C$_6$H$_4$(OH)CH$_2$OH, 2.4), 269(M$^+$-HOC$_6$H$_4$CH=CHCOO, 3.0), 164(HO-C$_6$H$_4$CH=CHCOOH$^+$, 100), 119(HOC$_6$H$_4$CH=CH$^+$, 5.4)

Thus, from the above data and spectra analysis, compound Ⅹ was confirmed to be a double aglycone glucoside consisting of salicyl alcohol, trans-*p*-hydroxycinnamic acid and glucose. If salicyl alcohol was lin