STUDIES ON THE CONSTITUENTS OF RADIX *PANAX GINSENG* C.A. MEYER

L. Hörhammer, H. Wagner and H. Lay.
Institute für Pharmazeutische Arzneimittlellehre der Universität München

The medicinal application of the drug Radix Ginseng, especially in Germany after the war, has motivated pharmacologists and pharmacognosists all over the world. Hence there has been intensive work and several reviews have been devoted to this subject (1, 2, 3, 4). Microscopic investigations on this drug, and its various commercial forms, through the detailed work of Weber (5), Schramm (6,7), Shimmonura and Kuro-Kawa (8) and Esdorn (9,10) may be considered to be complete. However the chemical work has a long since not yet achieved this state.

One finds in review articles mention of saponins or glycosides with saponin character, with specific reference to a watersoluble Panakilon, water insoluble Panakon and in addition Panaquilon or Panaxosid A and B. Tschirch (11) classifies Ginseng as a Saponin drug. Other components mentioned are essential oil (panacen), Panaxic acid and vitamin B_2. As far as the Saponin type of components are concerned they are attributed to Panax repens Maxim (Panax japonicum), a variant of the genuine Ginseng. This drug is not commercially available in Germany and is used as a substitute for Panax Ginseng in Japan. In 1932 the Japanse workers Murayama (12) and Aoyama (13) isolated a saponin-Panax saponin-from Panax repens Maxim. On hydrolysis they obtained a sapogenin which was identified as oleanolic acid through the work of Kotake and Kimoto (14) and Kirasato and Sone (15). Corresponding investigations on Panax Ginseng C.A. Meyer and Panax quinquefolius L., the American Ginseng, have not been carried out.

It is therefore understandable that there is a difference of opinion regarding the therapeutical worth of the drug-especially as the earlier pharmacological investigations yielded little definitive result. The more recent pharmacological investigations of Petkow (16, 17) did not alter the situation as no active principle with specific leads were found.

An objective of this work was therefore the isolation and renewed pharmacological investigation of pure compounds from the drug. In addition, chemical differences were sought between the various cultivated forms and the genuine Ginseng. It has been claimed that neither anatomic nor morphological characteristics are present to enable a differentiation of the drug according to its origin.

Starting material

As starting material a commercially available white chipped drug was used. It was declared as Panax Ginseng and according to the specifications of the importer (Fa. Nagelstein, Konstanz) was grown of genuine Korean stock in Japan. There was no doubt as to its identity and microscopic investigation excluded the possibility of Panax repens as the parent drug. It is assumed that the wild-growing

genuine Ginseng does not differ chemically from the cultivated ones.

Chemical investigations

Preliminary TLC examination with BAW as solvent indicated the presence of at least six antimony (V) Chloride-positive components. The alcoholic extract was worked up in two parallel ways. One aliquot was subjected to saponification and the nonsaponificable fraction further investigated for individual components. In the second procedure attempts were made to isolate the saponins and sapogenins. The experimental details were as follows:

1.) Ca. 800 g of the drug were exhaustively extracted in a Soxhlet and the extract concentrated in vacuum to a syrupy consistency, followed by saponification with hot 10% sodium hydroxide for 1 1/2 hours. After dilution with water and acidification the weakly acidic solution was extracted with ether. The etherextract was then successively extracted with 5% sodium bicarbonate solution and 5% aq. potassium hydroxide. The residual ether solution was evaporated and the residue chromatographed on a silica gel column. (Silica gel woelm).

The elution of the column was carried out according to the procedure of Stoll et al., (18, 19) using an (1:1) ether-petroleum ether mixture. The separation was effected with pure ether as solvent. All ten fractions, each 50 ml, were collected and each examined by TLC on Silica gel G. plates according to the method of Stahl (20) using chloroform or ether as the solvent and antimony (III) chloride as the sprayreagent. All the fractions with the exception of 6 and 7 indicated the presence of many compounds. Only fractions 6 and 7 contained a single compound which gave a strong red colour with antimony (III) chloride. Evaporation of the combined fractions and crystallisation of the residue yielded a white compound. Purification by chromatography on an aluminium oxide column (Aluminium oxide Woelm neutral activity 1.) followed by fractionated crystallisations yielded compound with a constant melting point of 137°C (Koflerblock). It gave a positive Liebermann-Burchard-reaction and its elemental anaylsis agreed with the formula $C_{29}H_{50}O$ (414. 69); Found C, 84. 71% and H, 12%, calculated C, 84.0% and H, 12. 15%. A comparison of the IR-and UV-spectra and the above data showed identity with β-sitosterol (22: 23-dihydrostigmasterol).

B-Sitosterol, belongs to the group of steroid sapogenins and occurs relatively widely especially in roots (21). As the presence of β-sitosterol in the ethanol and petroleumether extract in relatively larger concentration can be detected, it probably occurs predominantly in the free form.

Definitive pharmacological work on β-sitosterol is lacking. According to Best (22) and Schön (23) it is known that β-sitosterol is adsorbed in the intestine and leads to the lowering of the blood cholesterol level. Hence it has been suggested for the treatment of hypercholesteremia and myocardial infarct.

2.) For the preparation of the sapogenin fraction the drug was digested twice with 50% ethanol for 24 hours at 40°C in a water bath. The resulting extract was decanted after 48 hours and the residue extracted similary with ethanol for 96 hours. The combined extracts were concentrated, decolourised with charcoal and subjected to acid hydrolysis with 1% H_2SO_4 for 30 hours. The solution was brought to pH 6.5 and exhaustively extracted with ether, evaporated to dryness and subjected to TLC on silicagel G plate with ether as solvent.

The presence of four main components yielding a redbrown to red colouration with antimony (V) chloride. In addition, there were other spots with less characteristic brown colour reaction. The separation of this mixture was next attempted on a silicagel column using ether as eluent. Twelve fractions were separated and the contents examined by TLC and only fraction 5 showed homogenuity, corresponding to component A. This compound crystallised from alcohol as fine needles with a melting point of 305° (Kofler) and gave a positive Liebermann-Burchard reaction and an intensive red colour with antimony (V) chloride.

We compared this substance with other commonly occurring sapogenins-hederagenin, oleanolic acid, quillaic acid and ursolic acid and found that the isolated compound had the same Rf value and showed the same colour as oleanolic acid. (The

anthentic sample was kindly provided by Prof. Dr. R. Tschesche). For TLC we used ether, chloroform and the system suggested by Tschesche and coworkers (24) for triterpenes.

By comparison of melting points and the infrared spectra of the isolated compound and authentic oleanolic acid the identity was established.

In order to characterise the other compounds B, C and D we subjected the column fractions again to an acid hydrolysis in order to achieve total hydrolysis. However the results indicated that there was no change on hydrolysis. Hence this would indicate that other saponins are present in the drug. Though it could not be positively established that hederagenin was present in the mixture one can say with confidence that the sapogenin C and D are triterpene derivatives as they give a positive reaction with 2,6-ditert.-butyl-p-cresol. Brieskorn and Mahran (25).

Hence it could be shown by our work that one of the four sapogenins of Radix Panax Ginseng is identical with oleanolic acid, already shown to be present in Panax repens. In this connection it is interesting that the same oleanolic acid was also detected in some representatives of the family Aralia, as example e.g., Aralia montana (26) and Aralia chinensis var. glabrescens (27) and together with hederogenin in Aralia japonica (28). Kochetkov et al. have reported at the fifth Biochemical congress in Moskow, about the identity of oleanolic acid as the only genuine of four saponins of Aralia manschurica. On the basis of our work it is unlikely that Aralia manschurika and Panax Ginseng are identical.

In connection with the work on the activity of Crataegus extracts, the pharmacological effect of oleanolic acid was examined. According to Schimert (30), Böhm (31) and Ullsperger (32), oleanolic acid and other triterpenes are said to increase vitality and widen the coronary. These claims have been, however, disputed by Kuschke and Straub (33) and Bersin (34). Hence new work is necessary in order to establish whether the triterpenes have other pharmacological activity.

Hence it is too premature on the basis of the above reasons to pass judgement on the thereapeutic value of the Ginseng drug. One can also not definitely say whether the different commercially available forms of Ginseng are equivalent in a chemical sense.

Summary

β-Sitosterol and oleanolic acid were isolated in a pure form from Radix Panax Ginseng, the genuine Ginseng drug, by column chromatography on Silicagel and aluminium oxide (Woelm). TLC indicates the presence of at least three other triterpene sapogenins.

LITERATUR

1. B. SCHULZ, Dtsch. Apotheker-Ztg. **98**, 127 b (1958)
2. B. SCHULZ, Dtsch. Apotheker-Ztg. **99**, 303 (1958)
3. B. SCHULZ, Österr. Apotheker-Ztg. **12**, 272 (1959)
4. H. HOFMANN, U. HELD-HORNBOGEN and P. G. VOIGT, Pharmazie **5**, 560 (1950)
5. U. WEBER, Süddtsch. Apotheker-Ztg. **78**, 645, 657, 667 (1938)
6. G. SCHRAMM, Pharmaz. Ztg. **104**, 829 (1959)
7. G. SCHRAMM, Dtsch. Apotheker-Ztg. **98**, 1179 (1958)
8. SHIMMONURA, T. and H. KURO-KAWA Jour. Japanese Bot. **30**, 299 (1955)
9. I. ESDORN, Pharmazie **13**, 556 (1958)
10. I. ESDORN, Pharmazie **15**, 75 (1960)
11. A. TSCHIRCH, Handbuch d. Pharmakognosie II/2. S. 1517 Leipzig 1917, Verl. Chr. Herm. Tauchnitz
12. Y. MURAYAMA, J. Pharm. Soc. Japan **47**, 526 (1927)
13. S. AOYAMA, J. Pharm. Soc. Japan **50**, 1076 (1930)
14. M. KOTAKE and Y. KIMOTO, Sc. Papers Inst. Phys. Chem. Res. **18**, 89 (1932)
15. Z. KITASATO and Ch. SONE, Acta phytomim. **6**, 179, 223 (1932)
16. W. PETKOW, Arzneimittel-Forsch. **9**, 305 (1959)
17. W. PETKOW, Arzneimittel-Forsch, **11**, 418 (1961)
18. A. STOLL, E. SEEBECK and D. STAUFFACHER, Helv. chim. Acta **40**, 1205 (1957)
19. A. STOLL, E. ANGLIKER, F. BARFUSS, W. KUSSMAUL and I. RENZ, Helv. chim. Acta **34**, 1490 (1950)
20. E. STAHL, Chemiker Ztg. **82**, 323 (1958)
21. A. STOLL and E. JUCKER in K. Paech and M. V. TRACY Moderne Methoden der Pflanzenanalyse Bd. III, Springer Verlag, Berlin-Göttingen-Heidelberg (1955) S. 176.

22. C. H. BEST and C. H. DUNCAN, Ann. Int. Med. **45**, 614 (1956)
23. H. SCHÖN and P. ENGELHARDT, Arzneimittel-Forsch. **10**, 491 (1960)
24. R. TSCHESCHE, F. LAMPERT and B. SNAT ZKE, J. Chromatogr. **5**, 217 (1961)
25. C. H. BRIESKORN and G. H. MAHRAN, Naturwissenschaften **47**, 107 (1960)
26. A. W. van der HAAS, Ber. Dtsch. chem. Ges. **55**, 1054 (1922) and Rec. trav. chim. Pays-Bas **19**, 277 (1922)
27. S. KUWADA, J. Pharm. Soc. Japan **51**, 57 (1931)
28. A. WINTERSTEIN and G. STEIN, Hoppe-Seylers Z. physiol. Chem. **199**, 64 (1932)
29. N. K. KOCHETKOV, A. YA. KHORLIN, V. E. VASKOVSKII and V. E. ZHVIRBLIS, Vortrag auf dem V. Intern. Biochemiker Kongress in Moskau (1961)
30. G. SCHIMERT, Z. ges. exper. Med. **115**, 113 (1943)
31. K. BÖHM, Dtsch. Apotheker-Ztg. **94**, 890 (1954)
32. U. ULLSPERGER, Pharmazie **7**, 211 (1952)
33. H. J. KUSCHKE and H. STRAUB, Arch. exper. Path. Pharmakol. **226**, 547 (1955)
34. Th. BERSIN, Schweiz. Apotheker-Ztg. **95**, 403 (1957