114. *Sapogenins. Part VIII. The Sapogenin of Fuller's Herb.*

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The sapogenin obtained from *Saponaria officinalis* L. is shown to be gypsogenin and it is suggested that githagenin from corncockle is identical with this. The *iso*acetyl-lactone of gypsogenin has been oxidised in two stages to methyl hedragone lactone and a monobasic ketonic *acid* $C_{29}H_{44}O_5$.

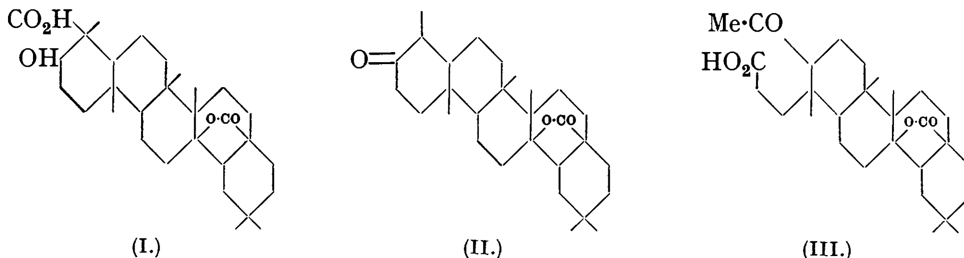
AMONGST plants containing comparatively large amounts of saponins those belonging to the *Caryophyllaceæ* are worthy of note; they include white soapwort (*Gypsophila* Spp.), fuller's herb (*Saponaria officinalis*), the corncockle (*Agrostemma githago*) and others.

In recent years the sapogenin obtained by the hydrolysis of *Gypsophila* saponin (gypsogenin) has been fully investigated and its constitution largely established by Ruzicka and Giacomello (*Helv. Chim. Acta*, 1936, **19**, 1136; 1937, **20**, 299); the sapogenin of the corncockle (githagenin) has been studied by Wedekind and Krecke (*Z. physiol. Chem.*, 1926, **155**, 122) and by Wedekind and Schicke (*ibid.*, 1929, **182**, 72; 1930, **190**, 1). We have now reinvestigated the sapogenin of saporubrin, the saponin of fuller's herb.

The crude saponin obtained by extracting the root with methyl alcohol or, better, with water, constitutes up to 20% of the root. It can be hydrolysed to the sapogenin without resort to heating under pressure, by the procedure found successful with quillaic acid (Elliott and Kon, J., 1939, 1130). The sapogenin crystallises somewhat reluctantly, but the acetate can easily be obtained pure and reconverted into the sapogenin, which is finally purified by sublimation in a high vacuum (compare Ruzicka, Giacomello, and Grob, *Helv. Chim. Acta*, 1938, **21**, 83). This sapogenin must already have been obtained, though not fully purified, by v. Schulz (*Arbeiten Pharm. Inst. Dorpat*, 1896, **14**, 82) and there is little doubt that it is identical with gypsogenin. The sapogenin itself is not easy to identify because the properties of different preparations vary somewhat, but the acetate is characteristic and gives rise to a well-defined methyl ester, a bromo-lactone and an *iso*acetyl-lactone, the identities of which appear to be beyond question.

The oxidation of the *iso*acetyl-lactone has been examined in order to compare its behaviour with that of the diacetyl-lactone of quillaic acid (Elliott and Kon, *loc. cit.*; Elliott, Kon, and Soper, preceding paper). The first product is the corresponding acid, which is readily deacetylated to the *lactone* of gypsogenic acid (I); this, like gypsogenic acid itself (below), is obtained in the form of *hydrate*, from which the *methyl* ester of the anhydrous acid is formed with diazomethane. Further treatment of the lactonic acid with chromic acid gives, exactly as with the corresponding derivatives of quillaic acid, a mixture of a neutral and an acidic product. The former is hedragone lactone (II) (Kitasato, *Acta Phytochim.*, 1934—5, **8**, 1), which has been characterised by its 2 : 4-*dinitrophenylhydrazone*;

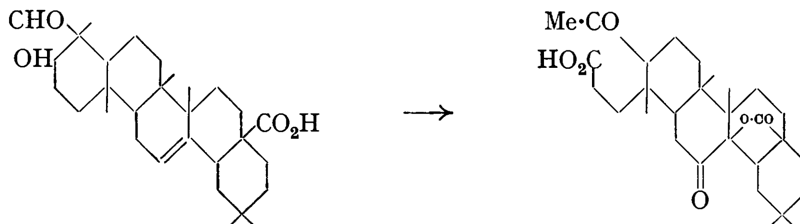
it shows a great resemblance to the diketo-lactone prepared by Elliott and Kon (*loc. cit.*). The *acid* formed at the same time consists of only one compound, which evidently represents the first stage in the oxidation of the lactone (II). It is a ketonic acid $C_{29}H_{44}O_5$ (III); its *methyl* ester shows the expected absorption spectrum with a weak band at 2925 A. (ε = 18·1) and readily forms a 2 : 4-*dinitrophenylhydrazone*.



(I.) (II.) (III.)

A critical review of Wedekind's work leads to the conclusion that githagenin must be identical with gypsogenin. Here again little importance can be attached to the melting point of the sapogenin and it is known to give low figures on combustion unless purified as above (compare Ruzicka and Giacomello, *loc. cit.*), but the melting points of the acetate, oxime and semicarbazone are comparable with the values for gypsogenin derivatives found by Karrer, Fioroni, Widmer, and Lier (*Helv. Chim. Acta*, 1924, **7**, 789); the isolation of a by-product of m. p. 328° (*iso*acetyl-lactone) in the acetylation of githagenin is particularly suggestive.

Wedekind and his collaborators carried out several degradation experiments on githagenin, but were unable to interpret their data owing to the scanty knowledge of triterpenes available at the time; their results are, however, in excellent agreement with the view expressed above. Thus, githagonolic acid, prepared by fusing githagenin with potassium hydroxide (Brandl, *Arch. Exp. Path.*, 1908, **59**, 245; Wedekind and Schicke, *Z. physiol. Chem.*, 1930, **190**, 1), is evidently identical with gypsogenic acid and is formed by a Cannizzaro reaction (CHO $\longrightarrow$ CO_2H); the melting points of the acid, its acetyl derivative, and their methyl esters (364°, 321°, 245° and 171°) agree well with those of Ruzicka and Giacomello's gypsogenin derivatives when it is remembered that the latter values are corrected (380°, 325°, 249—250° and 178—179°). Wedekind and Schicke's analysis figures agree perfectly with those required for gypsogenic acid $C_{30}H_{46}O_5$ (the acid itself was a hydrate) and its derivatives; it must be left an open question whether their githagoic acid was identical with this or not. The formation of githagic acid $C_{29}H_{42}O_6$ by the oxidation of githagenin with chromic acid is analogous to the production of the acid (III) and is formulated as follows:



It would thus appear that gypsogenin is widely distributed as a constituent of saponins occurring in the *Caryophyllaceæ* and may, indeed, be characteristic of them.

EXPERIMENTAL.

Saporubrin.—500 G. of powdered Rad. Saponariæ Rub. were boiled under reflux with 1·5 l. of methyl alcohol for 3 hours, the liquid filtered hot through a double layer of muslin (extract I), and the solid well pressed and washed with 250 c.c. of solvent. The extraction was repeated twice with 1 l. of methyl alcohol. 19 G. of almost colourless solid separated from

extract I overnight; the filtrate and the second and third extracts were evaporated, yielding 60, 30 and 27 g. of brown gum respectively.

Gypsogenin.—126 G. of crude saporubrin obtained as above were dissolved in 460 c.c. of hot water, and 334 c.c. of hydrochloric acid added, causing a crimson colour to develop. The solution was boiled in an oil-bath with mechanical stirring for 7 hours. The black solid was filtered off and extracted with hot water until coloured impurities were no longer removed; it was then dried, powdered, and extracted with ether (500 c.c.) in a modified Soxhlet apparatus (compare J., 1939, 1126). The solvent was changed after 3 hours, 6 hours and 16 hours. The first extract was concentrated to a small volume, and the solid filtered off and washed with ether, giving almost colourless gypsogenin, the filtrate being added to the second extract, which was similarly treated. In this way a total of 5·2 g. of crude gypsogenin, sintering at 267°, m. p. 276—277°, was obtained in addition to 10 g. of saporubrin.

For the preparation of gypsogenin the isolation of the saponin is not necessary and the procedure of Karrer, Fioroni, Widmer, and Lier (*loc. cit.*) can be used; the aqueous extract from 1 kg. of root is concentrated to 1 l., 340 c.c. of hydrochloric acid added, and the hydrolysis and extraction carried out as above. In this way 14·5 g. of nearly colourless gypsogenin were obtained in addition to 1·5 g. of very dark material. The latter could be purified by solution in about 150 c.c. of ether, shaking with 10 c.c. of N-potassium hydroxide, and decantation of the ether from the semi-solid, slimy precipitate of potassium salt, which was once more washed with ether. It was then covered with ether and shaken with dilute hydrochloric acid; the light brown ethereal solution was dried and shaken with norit. The filtered solution was yellow and gave an almost colourless solid on evaporation, which gave an excellent yield of the acetate on acetylation.

The crude sapogenin crystallised from alcohol on standing; from methyl alcohol it tended to separate in an amorphous form. The analytical specimen was prepared by allowing a solution of the pure acetate in methyl alcohol to stand with a slight excess of N-potassium hydroxide overnight. The potassium salt of gypsogenin soon began to separate in long needles and gypsogenin was recovered from it by shaking with ether and dilute acid; more was obtained by acidifying and extracting the mother-liquors from the potassium salt. The sapogenin was finally sublimed in a high vacuum at about 180°; at higher temperatures some decomposition was apparent. Even the sublimed material crystallised slowly from alcohol, forming felted needles, m. p. 269—270° after sintering (Found: C, 76·4; H, 10·0. Calc.: C, 76·6; H, 9·9%). The semicarbazone formed plates, m. p. 270—272° (decomp.), as found by Karrer *et al.* (*loc. cit.*). The acetate was readily obtained from the crude sapogenin with acetic anhydride and pyridine in the cold, forming fine needles sintering at 173°, m. p. 188—189° (Karrer *et al.*, *loc. cit.*), $[\alpha]_D + 79°$ ($c = 6·70$ in chloroform) (Found: C, 74·7; H, 9·5. Calc.: C, 74·9; H, 9·4%). It seems unlikely that the unsatisfactory m. p. of our acetate is due to an admixture of the acetyl-lactone, as found by Ruzicka and Giacomello (*loc. cit.*) not only on account of the method of preparation used by us, but also because the compound regenerated from the sodium salt and therefore presumably freed from lactone, still retained the same properties; no lactone could be isolated from the acetate after repeated fractional crystallisation from methyl alcohol. The methyl ester of the acetate was prepared with diazomethane and formed long needles, m. p. 191°, $[\alpha]_D + 80°$ ($c = 1·318$ in chloroform).

The acetate was converted into the bromo-lactone, which formed iridescent plates from methyl alcohol, m. p. *ca.* 180° (decomp.), and the *iso*acetyl-lactone, which crystallised in plates from acetic acid or, better, chloroform–methyl alcohol, m. p. 330—332°, in agreement with Ruzicka and Giacomello (*Helv. Chim. Acta*, 1937, **20**, 299). The *iso*acetyl-lactone is very readily prepared by merely keeping the crude genin with ten times its weight of acetic acid saturated with hydrogen bromide for 2 days. The rotation of our specimen was somewhat lower than the value recorded by Ruzicka and Giacomello, namely, $[\alpha]_D + 27°$ ($c = 2·629$ in chloroform).

Lactone of Gypsogenic Acid (III).—3·8 G. of the *iso*acetyl-lactone were suspended in 190 c.c. of "AnalaR" acetic acid and kept rapidly stirred while a solution of 1·37 g. of chromic acid in the minimum quantity of water, 68·5 c.c. of acetic acid, and 1·2 c.c. of sulphuric acid were dropped in during 5 hours. The excess of chromic acid was destroyed with methyl alcohol, the solvent distilled off under reduced pressure, and the residue treated with water and ether containing some chloroform. The ethereal solution was extracted with 200 c.c. of 10% potassium hydroxide solution to remove the acid formed; it gave on evaporation 0·7 g. of unchanged lactone. The alkaline solution was warmed on the steam-bath for 2 hours and acidified; an emulsion was then produced. This was shaken with ether, in which the new acid was virtually insoluble, and filtered, 2·7 g. of colourless solid being collected. It crystallised from

alcohol in needles, m. p. 353—355°, and was the *hydrate* of the acid (I) (Found: C, 71·5; H, 10·0. $C_{30}H_{46}O_5,H_2O$ requires C, 71·4; H, 9·6%); the *methyl* ester had m. p. 344—345° (Found: C, 74·4; H, 9·5. $C_{31}H_{48}O_5$ requires C, 74·4; H, 9·7%).

Oxidation of the Acid (I).—2 G. of the above acid in 200 c.c. of "AnalaR" acetic acid were mechanically stirred while 8·5 c.c. of Kiliani's solution were dropped in during 4½ hours. The oxidation was slow and an excess of chromic acid appeared to be present throughout; the excess was destroyed with methyl alcohol, the solvent distilled off under reduced pressure, and the residue extracted with ether and divided into neutral and acidic products; approximately equal amounts of these were isolated. The *acid* (III) crystallised from alcohol in thick prisms melting indefinitely at 270—280°, not altered by further crystallisation (Found: C, 73·6; H, 9·6. $C_{29}H_{44}O_5$ requires C, 73·7; H, 9·4%). The *methyl* ester formed thick transparent plates from methyl alcohol, m. p. 191—192° (Found: C, 73·9; H, 9·7. $C_{30}H_{46}O_5$ requires C, 74·1; H, 9·5%). The 2 : 4-*dinitrophenylhydrazone* formed yellow plates, m. p. 246—247° (Found: C, 64·6; H, 7·5. $C_{36}H_{50}O_8N_4$ requires C, 64·8; H, 7·6%).

The neutral product (II) separated from solvents in a gelatinous form unless it had been purified by percolation of its benzene solution through a column of activated alumina; thereafter it crystallised with great readiness, a behaviour characteristic of the diketo-lactone from quillaic acid (compare Elliott and Kon, *loc. cit.*); it formed needles from acetic acid or from chloroform–methyl alcohol, m. p. 298—301°, clearing at 304° (decomp.) (Found: C, 79·2; H, 10·2. Calc.: C, 79·0; H, 10·1%). It gave on treatment with bromine the sparingly soluble bromide, m. p. 283° (Kitasato, *loc. cit.*); the 2 : 4-*dinitrophenylhydrazone* separated from acetic acid in orange-yellow plates, m. p. 274—276° (decomp.) (Found: C, 68·1; H, 7·9. $C_{35}H_{48}O_6N_4$ requires C, 67·7; H, 7·8%).

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