

## CONSTITUTION OF CAMPANULIN

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Received June 15, 1961

THE isolation of the compound campanulin having the formula  $C_{30}H_{50}O$  from the leaves of *R. campanulatum*, *R. falconeri*, and *R. barbatum* has been reported in earlier publications from these laboratories.<sup>1-3</sup> The properties of the compound have already been described by us in detail.<sup>1</sup> The fact that it does not undergo acetylation (later confirmed by absence of hydroxyl frequencies in the I.R. spectrum) and does not form derivatives characteristic of carbonyl compounds (later confirmed by absence of carbonyl frequency in the I.R. spectrum), but undergoes isomerization under the influence of mineral acids has also been reported by us.<sup>2</sup> Further reactions and transformations leading to the structure of the compound are described in the present paper.

Since campanulin gives the reactions characteristic of steroids and triterpenoids,<sup>1</sup> its I.R. spectrum indicates the presence of a *gem*-dimethyl group (doublet at  $1360\text{ cm.}^{-1}$  and  $1390\text{ cm.}^{-1}$ ) and it occurs along with epifriedelanol<sup>1</sup> and friedelin<sup>4</sup> (which are definitely known to be triterpenoid compounds) it is also possibly a triterpenoid compound.

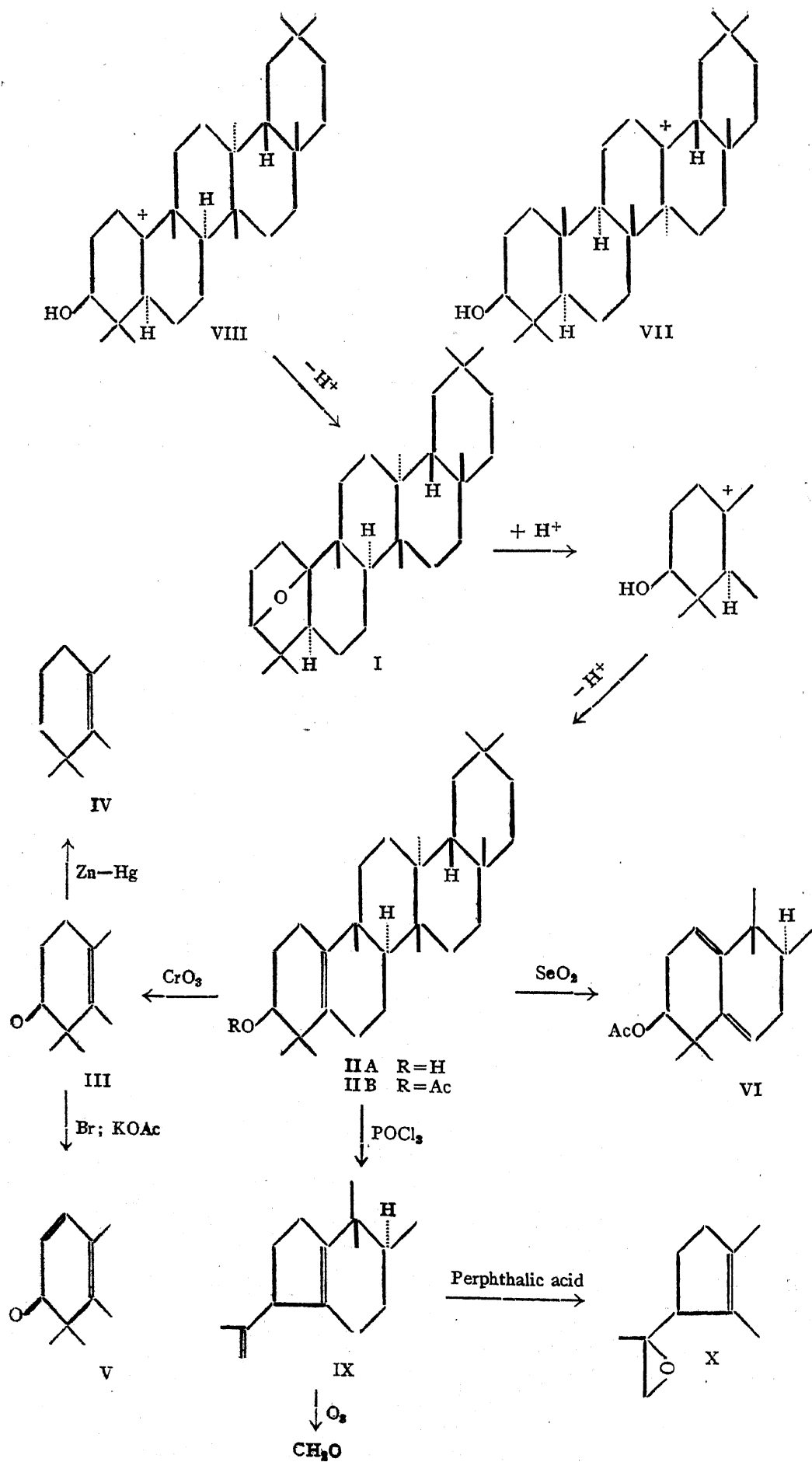
The compound has no unsaturation in it, since it does not give a colour with tetranitromethane in chloroform, does not react with perphthalic acid or undergo hydrogenation in presence of Adams PtO catalyst. The substance is free from methoxyl (micro-Zeisel) and was recovered after treatment with phosphorus oxychloride and pyridine (test for tertiary hydroxyl). These together with the earlier results regarding the nature of the oxygen function indicate that it is inert and possibly present as an oxide. In contrast to campanulin the product of isomerization with mineral acid underwent acetylation. Treatment of campanulin with *p*-toluene sulphonic acid-acetic anhydride, a reagent recommended by Ruzicka *et al.*<sup>5</sup> for splitting oxides, yielded a compound which was identical with the acetate of the isomerization product. These reactions show that campanulin should be a cyclic ether which opens out with mineral acids to give an alcohol and forms the alcohol acetate under the influence of *p*-toluene sulphonic acid-acetic anhydride. That this alcohol is a secondary alcohol was shown by its oxidation with

excess of  $\text{CrO}_3$  to a ketone ( $\text{C}_{30}\text{H}_{48}\text{O}$ ) (but not an acid) and by Clemmensen reduction of the ketone to a hydrocarbon,  $\text{C}_{30}\text{H}_{50}$ . The probable location of the carbonyl group at  $\text{C}_{(3)}$  of the ketone was indicated by a pink colour in the Zimmermann reaction,<sup>6</sup> while the  $\beta$ -orientation of the hydroxyl at  $\text{C}_{(3)}$  was indicated by the positive change in molecular rotation on acetylation ( $\Delta [\text{M}]_D = + 57^\circ$ ).<sup>7</sup> The properties of the alcohol, its acetate, the ketone and the hydrocarbon corresponded respectively with those of alnus-5 (10)-en-3 $\beta$ -ol, its acetate, alnus-5 (10)-en-3-one and alnus-5 (10)-ene described by Beaton *et al.*<sup>8</sup> in almost all essential details. That the alcohol (II A), its acetate (II B) and the ketone (III) were really what they were suspected to be was confirmed by reaction of the alcohol acetate with  $\text{SeO}_2$  to give alnusa-1 (10):5-dien-3 $\beta$ -yl acetate (VI)<sup>9</sup> and by the ketone yielding alnusa-1:5 (10)-dien-3-one (V) on treatment with bromine followed by potassium acetate.<sup>8</sup>

Final confirmation of the identity of the ketone was achieved by a direct mixed m.p. determination with a sample of alnus-5 (10)-en-3-one, kindly supplied by Dr. McLean from Professor Spring's collections.

The identity of the ketone (III) and of the alcohol (II A) having thus been established, the structure of the original compound campanulin follows from the following considerations. In contrast to the alcohol (isomerization product) which gives a positive reaction with tetranitromethane, campanulin does not give any colour with this reagent. This shows that the appearance of the (5:10) double bond is a concomitant of the opening of the oxide ring to give the alcohol. Since one of the points of attachment of the oxygen is  $\text{C}_{(3)}$ , the other would be either at  $\text{C}_{(5)}$  or  $\text{C}_{(10)}$ . Since the I.R. spectrum of campanulin shows a band at  $9.1 \mu$  which is considered to be characteristic of tetrahydrofurans, while a band at  $10.2\text{--}10.3 \mu$  characteristic of trimethylene oxides is missing in the spectrum (*see* Borrow and Searles<sup>10</sup>), the second point of attachment of the oxide seems to be  $\text{C}_{(10)}$  and not  $\text{C}_{(5)}$ . This is perhaps to be expected from considerations of relative stability and also because the large majority of the known natural oxides have the oxygen atom present in a 5-atom ring. Hence campanulin can be represented by structure (I) in which the configuration of H at  $\text{C}_{(5)}$  is assumed to be the same as in all well-known triterpenes.

In the course of their work on the constitution of alnusenone, Spring and coworkers<sup>9</sup> have suggested a scheme of biogenesis according to which  $\beta$ -amyrin, taraxerol, alnusenone and friedelin can all be derived from a common hypothetical precursor (VII) by changes involving the loss of a proton with or without being preceded by  $\alpha$  shift of a methyl or hydrogen, each of which may take place only once or may be repeated twice or even more as necessary.



One of the intermediate stages in this scheme is represented by the structure (VIII) and it is quite conceivable that campanulin arises from this by loss of a proton from the  $C_3$ -OH.

It may be mentioned by the way that the compound (II A) underwent the retro-pinacolinic rearrangement on treatment with phosphorus oxychloride giving rise to (IX) which was characterized by its reaction with perphthalic acid to give an epoxide (X) and by the formation of formaldehyde on ozonolysis. The I.R. spectrum of (IX) included strong and sharp bands at  $1634\text{ cm.}^{-1}$  and  $890\text{ cm.}^{-1}$  which are characteristic of a vinylidene group,  $\text{>C} = \text{CH}_2$ . This reaction has not been described in the literature so far and is therefore given here.

#### EXPERIMENTAL

For properties and analysis of campanulin *see* p. 100 of ref. 1. For the isomerization reaction yielding alcohol (II A) *see* p. 246 of ref. 2.\* The acetate of the alcohol, prepared by heating with pyridine and acetic anhydride for 2 hours at  $100^\circ$ , crystallized from chloroform-acetone as colourless plates, m.p.  $286\text{--}88^\circ$ .  $[\alpha]_D^{20} = -17.6^\circ \pm 2^\circ$  ( $c = 0.920$  in chloroform). Found: C, 81.7; H, 11.1.  $C_{32}H_{52}O_2$  (II B) requires: C, 82.0; H, 11.2%.

*Preparation of the acetate (II B) directly from campanulin.*—A mixture of campanulin (0.1 g.), acetic anhydride (10 ml.) and *p*-toluene sulphonic acid (50 mg.) was heated at  $115^\circ$  for 45 minutes. It was cooled, ice-cold water (30 ml.) was added and left in the ice-chest overnight. The precipitate that separated was filtered, washed, dried and crystallized thrice from chloroform-acetone when shining colourless plates were obtained, m.p.  $285\text{--}87^\circ$  (80 mg.).  $[\alpha]_D^{20} = -14.8^\circ \pm 3^\circ$  ( $c = 0.732$  in chloroform). Found: C, 82.3; H, 11.6.  $C_{32}H_{52}O_2$  (II B) requires: C, 82.0; H, 11.2%. It gave a yellow colour with tetranitromethane in chloroform. Mixed m.p. with the sample prepared from (II A) was undepressed.

*Preparation of ketone (III).*—A solution of alcohol (II A) in benzene was treated with a 2% solution of chromic acid in acetic acid added dropwise with continuous shaking for a total period of 15 hours, an excess of the reagent being maintained throughout. Most of the solvents were removed under reduced pressure and the residual liquid was diluted with dilute sulphuric acid and extracted with ether. The ethereal extracts were washed with sodium carbonate solution and then with water, dried and the

\* The molecular formula  $C_{30}H_{48}O$  assigned to this alcohol in ref. 2 has to be revised to  $C_{30}H_{50}O$  to conform to the formula of campanulin given in ref. 1.

solvent removed. Crystallization of the residue twice from chloroform-acetone yielded shining colourless prismatic plates, m.p. 250–51°. It gave a yellow colour with tetranitromethane in chloroform. A pink colour was obtained in the Zimmermann test.  $[\alpha]_D^{20} = -73.4^\circ \pm 2^\circ$  ( $c = 1.062$  in chloroform). Found: C, 84.8; H, 12.0.  $C_{30}H_{48}O$  (III) requires: C, 84.8; H, 11.4%. Mixed m.p. with an authentic sample of alnus-5 (10)-en-3-one (m.p. 251–52°) (from Professor Spring's collections) was un-depressed.

The 2:4-dinitrophenylhydrazone, prepared in the usual way, crystallized from chloroform-alcohol as shining orange-red needles, m.p. 269–70°. Found: N, 10.1.  $C_{36}H_{52}O_4N_4$  requires: N, 9.3%.

*Preparation of hydrocarbon (IV).*—To a boiling solution of ketone (III) (0.3 g.) in alcohol (200 ml.) and benzene (20 ml.) was added amalgamated zinc (65 g.), followed by conc. hydrochloric acid (2 ml.). Refluxing was continued for 36 hours with intermittent addition of further small quantities of acid and/or alcohol as and when required. The light yellow syrupy product which was obtained by working in the usual manner was subjected to vacuum sublimation (0.4 mm. pressure). Three fractions were obtained at 150–80°, 200–35° and 240–60° respectively. The first and the last fractions were amorphous. The middle fraction (90 mg.), on crystallization first from chloroform-alcohol and then from chloroform-acetone yielded thin colourless plates, m.p. 225–26° (23 mg.).  $[\alpha]_D^{20} = -35.6^\circ \pm 1^\circ$  ( $c = 0.758$  in chloroform). Found: C, 86.8; H, 12.2.  $C_{30}H_{50}$  (IV) requires: C, 87.7; H, 12.3%. It gave a yellow colour with tetranitromethane in chloroform.

*Preparation of dienone (V) from (III).*—This was prepared according to the method of Beaton *et al.*<sup>8</sup> using bromine in acetic acid followed by potassium acetate. The product which was impure was chromatographed over alumina. The petroleum ether-benzene (19:1 and 9:1) eluates yielded unconverted (III) (m.p. 249–52°). The petroleum ether-benzene (4:1 and 1:1) eluates yielded from chloroform-methanol shining yellow plates, m.p. 215–16°. Found: C, 84.7; H, 11.7.  $C_{30}H_{46}O$  (V) requires: C, 85.3; H, 11.0%.

*Selenium dioxide oxidation of acetate (II B) to (VI).*—The oxidation was carried out as described in the literature.<sup>9</sup> The product which was purified by chromatography over alumina crystallized from chloroform-methanol as shining colourless plates, m.p. 206–07°.  $[\alpha]_D^{20} = +118.7^\circ \pm 2^\circ$  ( $c = 1.365$  in chloroform). Found: C, 82.0; H, 11.6.  $C_{32}H_{50}O_2$  (VI) requires: C, 82.3; H, 10.8%. It gave a deep orange colour with tetranitromethane.

*Preparation of (IX).*—A solution of the alcohol (II A) (1 g.) in pyridine (400 ml.) was treated with phosphorus oxychloride (55 ml.) drop by drop with stirring. The reaction mixture was left at room temperature for 16 hours and then heated on a steam-bath for 30 minutes. The resulting dark brown solution was cooled and poured slowly on crushed ice. The yellow aqueous solution was extracted with petroleum ether ( $8 \times 100$  ml.), the extract was washed with water, then with 2 N hydrochloric acid and finally with water till neutral. It was dried and filtered through a column of alumina (3 g.). Evaporation of the filtrate gave a reddish yellow residue, which on crystallization thrice from chloroform-acetone yielded shining colourless plates, m.p.  $195-97^\circ$  (0.48 g.).  $[\alpha]_D^{25} = -53.0^\circ \pm 1^\circ$  ( $c = 1.464$  in chloroform). Found: C, 87.9; H, 12.5.  $C_{30}H_{48}$  (IX) requires: C, 88.1; H, 11.8%. It gave a deep yellow colour with tetranitromethane in chloroform.

*Preparation of epoxide (X).*—A solution of (IX) (50 mg.) in chloroform (5 ml.) was treated at  $-10^\circ$  with excess of a solution of monoperphthalic acid in chloroform. The mixture was left in the ice-chest for two days and worked up in the usual manner. The product crystallized from chloroform-acetone as colourless nodules, m.p.  $209-11^\circ$ . Found: C, 84.2; H, 11.9,  $C_{30}H_{48}O$  (X) requires: C, 84.8; H, 11.4%.

*Ozonolysis of (IX)—detection of formaldehyde.*—A solution of (IX) (0.4 g.) in dry chloroform (30 ml.) was treated at  $-10^\circ$  with ozonised oxygen (1.5%  $O_3$  content) (ca. 100 ml. per minute) for 25 minutes. After attaining room temperature, the solution was treated with acetic acid (3 ml.) and portionwise with zinc dust (0.6 g.) over 30 minutes with stirring. After 1 hour's stirring, the solution was filtered and the filtrate washed with water ( $3 \times 15$  ml.). The aqueous washings were combined, adjusted to pH 7.0 by the addition of solid sodium bicarbonate when a colourless precipitate separated, which was filtered. The clear, colourless filtrate gave with saturated aqueous solution of dimedone a colourless precipitate which crystallized from alcohol as colourless needles (10 mg.), m.p.  $192-93^\circ$ , undepressed by admixture with authentic formaldehyde-dimedone compound.

#### SUMMARY

Experiments leading to the establishment of the constitution of campanulin are described and discussed.

#### ACKNOWLEDGMENTS

The authors express their gratitude to Professor Spring and Dr. J. McLean of Royal College of Science and Technology, Glasgow, for kindly supplying

the reference sample of alnus-5 (10)-en-3-one and Professor T. R. Govindachari of the Presidency College, Madras, for taking the infra-red spectra of the several compounds discussed in this paper. We thank Mr. R. V. Krishna Rao for microanalyses.

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## EPILOGUE

The work described in this article including the experiments, observations, interpretations and conclusions and also biogenetic relationships, formed part of a thesis submitted by one of the authors (K. S. M.) for the D.Sc. degree of the Andhra University, Waltair, in August 1960. The thesis was approved in December 1960 and a copy of the same was deposited in the INSDOC Library at New Delhi in the same month. It has become necessary to state these facts because in the number of the *J. Chem. Soc.* (Feb. 1961) which has been received in our library a few days back, there is an article from the Hong-Kong University (H. R. Arthur and Miss W. H. Hui, p. 551) which deals obviously with the same substance as our own, though the experiments that they have described do not tally with our own. In their earlier publication (H. R. Arthur, C. M. Lee and C. N. Ma, *J. Chem. Soc.*, 1956, 1461) describing the isolation of their compound, they reported the specific rotation as  $-80^\circ$ , but have completely reversed the sign (to

+ 74.8°) in their present paper. Since our own substance had a specific rotation of + 75.2° we were misled into concluding that we were not dealing with the same substance as theirs and into believing that there was no special urgency about the publication of our own findings, though our work was completed long before August 1960 (the date of submission of the thesis mentioned above).