Tetrahedron, 1967, Vol. 23, pp. 2557 to 2561. Pergamon Press Ltd. Printed in Northern Ireland

CORYMBOSIN, A GLUCOSIDE FROM *TURBINA CORYMBOSA**

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(*Received in USA* 8 *August* 1966; *accepted for publication* 11 *November* 1966)

Abstract—A minor glucoside, corymbosin, has been isolated from *Turbina corymbosa* and the structure and partial stereochemistry of its aglucone, corymbositin, established.

From the seeds of *Turbina corymbosa*, a glucoside, turbicoryn,[1] and a diterpenic alcohol, corymbol,[2] have been described. From the same seeds a minor glucoside, corymbosin, could be isolated.

Corymbosin (I) is an isomer of turbicoryn, with a molecular formula

$$C_{29}H_{50}O_{11}\cdot\frac{1}{2}H_2O$$

Upon acetylation it gives a nona-acetate, $C_{47}H_{68}O_{20}\cdot 2MeOH$, from which the glucoside can be regenerated by saponification. Enzymatic hydrolysis of corymbosin gives a mole of glucose and the aglucone, corymbositin (II), with a molecular formula $C_{23}H_{40}O_6\cdot 1\frac{1}{2}H_2O$. Acetylation of corymbositin with acetic anhydride and pyridine at 92° yields an hexa-acetate, but if acetylation is carried out at room temperature a penta-acetate is obtained. The NMR spectra are in good agreement with the analytical data for these two acetates.

Aromatization of corymbosin with selenium gives a mixture of 1-methyl- and 1,7-dimethylphenanthrene which were identified by their UV and NMR spectra, m.ps, picrates and trinitrobenzene adducts. This shows that corymbosin and turbicoryn are structurally related, and have the same fundamental nucleus.

During periodic acid degradation, corymbosin uses three moles of periodic acid and a ketone III is obtained; also, two moles of formic acid and one of formaldehyde are produced, thus establishing the structure of the polyhydroxylated side chain as being the same as in turbicoryn (1).

The IR spectrum of ketone III shows max at 3530 and 3430 cm^{-1}, corresponding to primary alcohols; at 1740 cm^{-1}, corresponding to a 5-membered ring ketone, and a strong absorption between 1100 and 1000 cm^{-1}, corresponding to hydroxymethylene groups. The band at 3430 cm^{-1} is reduced on dilution in accordance with intermolecular association. The NMR spectrum shows two C-Me groups at 1·10 and 1·00 ppm. The signals between 1·9 and 2·6 ppm, which integrate for 3 protons, correspond to the vicinity of the keto group

$$\left(\begin{array}{c} | \quad\quad\quad\;\; | \\ -\underset{H}{C}-\underset{O}{\overset{\|}{C}}-\underset{H}{C}-\underset{OH}{CH_2} \end{array}\right).$$

This ketone III is unstable and gives positive Tollens and Benedict tests. It readily loses formaldehyde by treatment with hydrochloric acid giving a new ketone IV, $C_{19}H_{30}O_2$, with a mol. wt. of 290 (M.S.). All these properties of ketone III are similar

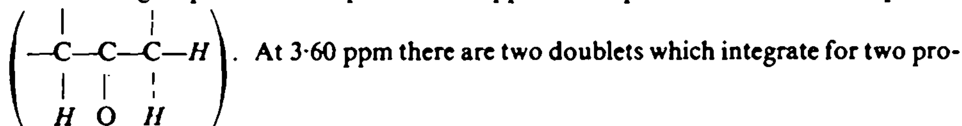
* Contribution No. 235 from the Instituto de Química de la Universidad Nacional Autónoma de México, México 20, D.F.

[1] M. C. Pérezamador, F. García Jiménez, J. Herrán and S. E. Flores, *Tetrahedron* 20, 2999 (1964).

[2] M. C. Pérezamador and F. García Jiménez, *Tetrahedron* 22, 1937 (1966).

to those of the corresponding degradation product of turbicoryn and indicate the presence of a hydroxymethylene α to the keto group.

Ketone IV shows in the IR spectrum bands at 3550 and 3470 cm^{-1}, corresponding to OH groups; this last band is modified on dilution indicating intermolecular association. The CO band appears at 1740 cm^{-1} and at 1000 cm^{-1}, a band corresponding to a primary alcohol. In the NMR spectrum two peaks at 1·07 and 0·97 ppm indicate the presence of two C–Me groups. There is a double signal of an A–B system (almost A_2) at 1·97, with a separation of 1 c/s, corresponding to two protons α to the CO group. The multiplet at 2·40 ppm corresponds to the other α proton

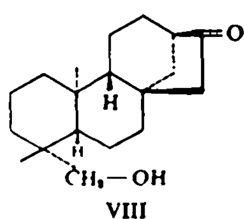


. At 3·60 ppm there are two doublets which integrate for two protons; these signals are characteristic of a hydroxymethylene group with hindered rotation because of steric interactions.[2]

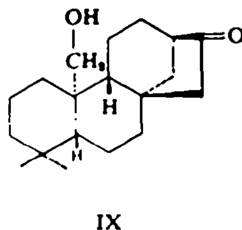
The sterical hindrance of the —CH_2OH is indicated too by the slow oxidation of IV with Jones reagent. In this reaction a mixture of the ketoaldehyde VI, $C_{19}H_{28}O_2$, and the ketoacid VII, $C_{19}H_{28}O_3$, which were separated by chromatography on silica gel, was obtained.

The Wolff-Kishner reduction of ketone IV gives an alcohol V, $C_{19}H_{32}O$, with a mol. wt. of 276 (M.S.) whose NMR shows the signal corresponding to the —CH_2OH at 3·48 ppm.

The mass spectrum of keton IV* shows a fragmentation pattern similar to the one of its isomer ketone (M-290) in the turbicoryn series,[1] and the CD† curves of both ketones are practically superimposable. The similarity in the mass spectra indicates the presence of a similar carbon skeleton and the CD curves demonstrate that the stereochemistry of the ring fusions C/D and B/C is the same in both compounds. Having in mind the biogenetic rule for the stereochemistry of the diterpenes of all *trans-anti* ring fusion, the stereochemistry of ketone IV must be similar to that of its isomer, which is logical, since both compounds are found in the same plant and are closely related substances. Accordingly, the two more likely possibilities for the structure and absolute configuration of ketone IV are the following:



VIII



IX

Both of these compounds have been described.[3,4] Direct comparison by m.ps, NMR and IR spectra showed that our product is identical with compound VIII.‡

* We wish to thank Dr. E. Lederer for the determination of this mass spectrum.

† We wish to thank Dr. G. Ourisson for the C. D. determination.

‡ We wish to thank Dr. P. R. Jefferies of the University of Western Australia, Nedlands, W.A., for the sample of 16-oxo-17-nor-(−)-kauran-19-ol that he kindly sent us.

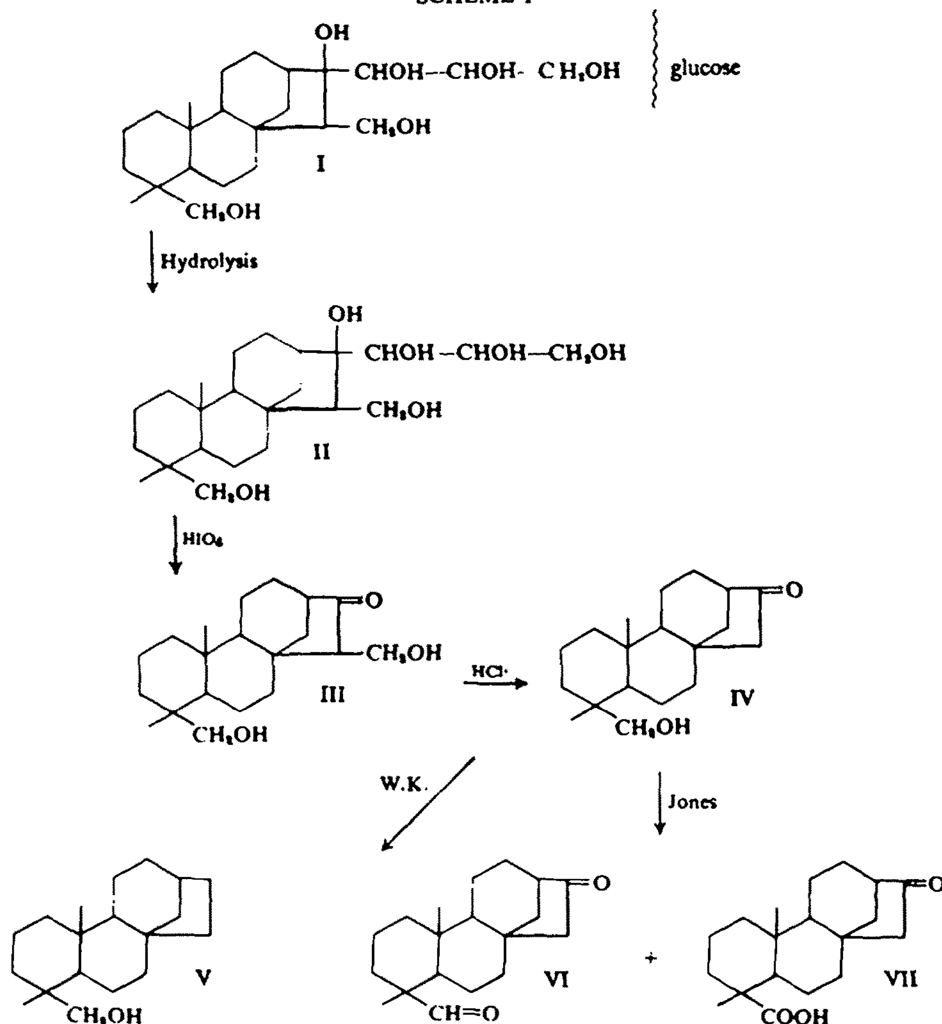
[3] C. A. Henrick and P. R. Jefferies, *Austral. J. Chem.* 17, 915 (1964).

[4] S. Masamune, *J. Am. Chem. Soc.* 86, 289 (1964).

Therefore, we propose structure I for corymbosin and structure II for corymbositin (scheme 1 shows the degradation reactions of corymbosin).

It is very probable that the stereochemistry of the hydroxymethylene group in C-15 of corymbosin is the same as that in turbicoryn because of the closely related structures of these compounds, but this point and the stereochemistry of the side chain deserve further study.

SCHEME 1



EXPERIMENTAL*

Isolation of corymbosin (I). The ethanolic extract[1] (50 g) from the seeds of *T corymbosa* was dissolved in 100 ml MeOH and 900 ml water were added. Treatment with lead subacetate, filtration,

* M.ps were determined on a Kofler block and are uncorrected. IR spectra were run on a Perkin-Elmer model 21 spectrophotometer in $CHCl_3$. NMR spectra were determined by Mr. Eduardo Díaz on a Varian A-60 spectrometer in $CDCl_3$ containing TMS as internal standard. The mass spectra were run by Mr. Eduardo Cortés on a RMU-6D Hitachi Perkin-Elmer double focus spectrometer at 75 eV. Microanalyses were determined by Dr. Franz Pascher, Bonn, Germany. Silica Gel G Merck was used for chromatoplates which were developed with ceric sulphate.

precipitation of excess lead subacetate with H_2S and again filtration gave a clear soln which was evaporated to 50 ml. The soln was allowed to stand 72 hr at 0°. The ppt formed was filtered off and washed first with water and then with acetone. The solid (15 g) was recrystallized from MeOH. The first crop was almost pure turbicoryn (12 g). The mother liquors were formed by a mixture of almost equal parts of turbicoryn and corymbosin (chromatoplate run with butanol–AcOH–water 5:1:4). Repeated crystallizations of this mixture from isopropanol, yielded 0·900 g of I, which is less soluble than turbicoryn in most solvents. The analytical sample was crystallized from MeOH–water, m.p. 305–306°, $[\alpha]_D^{25}$ −59·2° (c = 3; pyridine). (Found: C, 59·08; H, 8·65; O, 32·02. $C_{33}H_{54}O_{11}\cdot\frac{1}{2}H_2O$ requires: C, 59·67; H, 8·80; O, 31·53%.)

Acetylation of corymbosin

Corymbosin (100 mg, m.p. 305–306°) was acetylated with Ac_2O (1 ml) and anhyd pyridine (1 ml), by heating 3 hr at steam bath temp (92°). The soln was poured into water and the ppt was filtered off washed twice with 5% HCl and then with water. The acetate (148 mg) gave one spot on a chromatoplate run with AcOEt. For the analytical sample it was crystallized 3 times from MeOH–AcOEt, m.p. 219–220°, $[\alpha]_D^{22°}$ −35·0° (c = 2·9; $CHCl_3$). (Found: C, 57·40; H, 7·77: $C_{47}H_{64}O_{20}$.2MeOH requires: C, 57·86; H, 7·53.

Saponification of the nona-acetate. The acetate (100 mg, m.p. 219–220°) was saponified under the usual conditions. The product (55 mg, m.p. 304–306°) was crystallized from MeOH–water and had an IR spectrum, m.p. and mixed m.p. identical with corymbosin.

Enzymatic hydrolysis of corymbosin

Corymbosin (1 g, m.p. 305–307°) in 20 ml MeOH, was evaporated to approx. 10 ml and diluted to 1000 ml with distilled water. To this soln, 100 ml acetate buffer pH 4·9 was added, which contained the emulsion from 10 g pulverized almonds, and then incubated for 78 hr at 37°. The reaction mixture was concentrated *in vacuo* to 50 ml. The ppt was filtered off and the aglucone II was dissolved from the wet filter with acetone (200 ml.) The acetone soln was dried (Na_2SO_4), filtered and concentrated under N_2 until II (600 mg; 83·5% of theoretical) crystallized out. These crystals gave one spot on a chromatoplate run with AcOEt–MeOH (1:1). The analytical sample was crystallized from MeOH–water and dried *in vacuo* the product being highly hygroscopic, m.p. 220–222°; end absorption at 206 mμ (MeOH), ε = 10.5. (Found: 63·20; H, 9·17; O, 27·30. $C_{33}H_{48}O_6$. $1\frac{1}{2}H_2O$ requires: C, 62·85; H, 9·87; O, 27·28.)

Identification of glucose. The aqueous soln of the enzymatic hydrolysis, after separation of the aglucone, was concentrated *in vacuo* to 25 ml, phenylhydrazine hydrochloride (250 mg) and AcONa (250 mg) were added and the soln was heated on the steam bath for 10 min. The osazone obtained was compared under the microscope with the osazone of an authentic sample of glucose. Both were identical.

The glucose was also identified by paper chromatography with butanol–AcOH–water 5:1:4 and developed with oxalic acid 0·1M and aniline.

Acetylation of corymbositin

(a). *Hexa-acetate.* Corymbositin (100 mg, m.p. 220–222°) was acetylated as for corymbosin nona-acetate, yielding 160 mg of the hexa-acetate. On a chromatoplate run with 3% Chf–MeOH one main spot was observed with a more polar minor spot. The analytical sample was purified by TLC, followed by crystallization from MeOH–water and showed m.p. 196–197°, (Found: C, 61·62; H, 7·62. $C_{33}H_{48}O_{12}\cdot 1H_2O$ requires: C, 61·56; H, 7·97%.)

(b). *Penta-acetate.* Corymbositin (100 mg, m.p. 220–222°) was acetylated as for corymbosin nona-acetate, but at room temp for 24 hr, yielding 150 mg of the penta-acetate. On a chromatoplate there was one main spot and another due to the less polar hexa-acetate. The analytical sample was obtained by TLC and crystallization from MeOH–water; m.p. 152–153°. (Found: C, 62·41; H, 8·16. $C_{33}H_{44}O_{11}\cdot\frac{1}{2}H_2O$ requires: C, 62·73; H, 8·13%.)

Periodic acid degradation of corymbositin

(a). *Moles of periodic acid used.* To a soln of 81·2 mg corymbositin (m.p. 220–222°) in 15 ml MeOH, 50 ml of an aqueous soln of H_5IO_6 (6·8385 g in 1000 ml) was added and allowed to react 12 hr

at room temp. At the same time a blank was prepared. To both flasks 5 g KI in 50 ml water and 10 ml 1:10 H_2SO_4 were added and titrated with 0·1N $Na_2S_2O_3$. The difference was 10·5 ml which corresponds to 2·58 moles of HIO_4.

(b) *Moles of formic acid produced.* Corymbositin (97·3 mg, m.p. 220–222°) was oxidized with 3 moles of periodic acid. The resulting soln was diluted with water (20 ml) and potentiometrically titrated with 0·1N NaOH using 4·45 ml which corresponds to 1·95 moles formic acid.

(c) *Moles of formaldehyde produced.* Corymbositin (96·3 mg, m.p. 220–222°) was subjected to oxidation with 3 moles periodic acid. The reaction mixture was steam distilled and to 250 ml of distillate, 50 ml of 50% alcoholic aqueous solution with 1% dimedone was added. It was allowed to stand 12 hr and the ppt was filtered off (53·6 mg, m.p. 189–190°). No depression in the m.p. was observed when mixed with an authentic sample of formaldimedone. These 53·6 mg correspond to 0·80 moles of formaldehyde.

Periodic acid oxidation of corymbositin

Corymbositin (0·278 g, m.p. 220–222°) in 20 ml MeOH, was treated with 0·550 g periodic acid in 4 ml water. The mixture was stirred 1 hr and allowed to stand 12 hr at room temp. The soln was poured into 20 ml water and neutralized with $NaHCO_3$. It was extracted with ether and after washing the extract with water, the solvent was evaporated to dryness, yielding a white foamy product (0·200 g), which could not be induced to crystallize. It gave one spot on a chromatoplate run with 35% AcOEt–benzene and corresponds to III as shown by the NMR and IR spectra. A good analysis could not be obtained for this product.

Ketone III gave positive Benedict and Tollens tests. On treatment with 8% HCl, formaldehyde was produced, identified by reaction with dimedone and isolation of the adduct (m.p. 189–190°). This ketone was oxidized by air giving a product which liberated I_2 when treated with KI soln.

Ketone III (200 mg) was dissolved in 20 ml of 50% aqueous MeOH with 8% HCl and refluxed for 4 hr giving after cooling a white crystalline ppt (150 mg, m.p. 132–137°). On a chromatoplate run with 60% AcOEt–hexane, one main spot and a minor spot, due to a less polar substance, were observed. The product was purified by TLC run with the same mixture of solvents and extracted with AcOEt–MeOH 4:1. The analytical sample was crystallized from acetone–water and had m.p. 153–154° and 156–157°. No depression was observed in a mixed m.p. with an authentic sample of 16-oxo-17-nor-(−)-kauran-19-ol. M, 290 (M.S.). (Found: C, 76·24; H, 10·17; O, 13·35. $C_{19}H_{30}O_2 \cdot \frac{1}{2}H_2O$ requires: C, 76·21; H 10·44; O, 13·36%.)

The semicarbazone had m.p. 278–280° and the 2,4-dinitrophenylhydrazone, 250–251°.

Alcohol V. The semicarbazone of ketone IV (1;0 mg) was subjected to Wolff-Kishner reduction with 0·5 ml of hydrazine and 5 mg Na dissolved in 2·5 ml ethylene glycol. The mixture was heated for 2 hr at 205–210° in a sealed glass tube. The product crystallized in the tube on cooling. The entire contents of the tube were poured into water and the white crystalline product (78 mg, m.p. 123–127°) filtered off. It gave one spot on a chromatoplate run with hexane–AcOEt 2:3. Purified by sublimation at 130°/0·05 mm it melted at 130–132° and had M, 276 (M.S.); ν_{max} 3650, 3395 and 1009 cm^{-1}; RMN, 0·95 (C-4 Me), 0·99 (C-10 Me) and 3·58 ppm (C—*C*H_2—OH).

Aldehyde VI *and acid* VII. To ketone IV (250 mg) in 50 ml acetone were added dropwise and with stirring, 0·5 ml of Jones reagent.[4] The mixture was neutralized with solid $NaHCO_3$ and filtered. The clear acetone soln was evaporated and the residue chromatographed on silica gel G-922 eluting with 65% AcOEt–hexane. The first fraction (175 mg, m.p. 130–132°) was aldehyde VI, ν_{max} 2850 and 2680 (*H*—C— O), 1736 (C=O) and 1693 cm^{-1} (H—*C*=*O*); NMR, 1·05 and 1·17 (C-methyl groups, 6H), 1·90 (—*C*H_3—C=O, s), 2·38 (—C—C=O, m; with *H* on the first C) and 9·42 ppm (*H*—C—O, s); M, 288 (M.S.).

The second fraction was the acid VII, m.p. 230–232°.

[4] A. Bowers, T. G. Halsall, E. R. H. Jones and A. J. Lemin, *J. Chem. Soc.* 2555 (1953).