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Biomimetic synthesis of cannabispiran[1]

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Summary. Total synthesis of cannabispiran (**1**) was accomplished by a biomimetic-type cyclization of the bibenzyl **2**, using $K_3Fe(CN)_6$ or $MoOCl_4$.

Cannabispiran (**1**) and related compounds are structurally related to some synthetic estrogen-potentiating agents[2]. Thus, it was proposed that they might exert a similar effect in marihuana, which is known to have estrogenic activity[2]. Furthermore, cannabispiran (**1**) itself is likely to be biogentically derived from the bibenzyl **2** which was recently reported[3] to have plant growth retarding activity. A similar activity by cannabispiran (**1**) cannot be discounted.

Because of the paucity of cannabispiran (**1**) in the natural source, it was necessary to develop a method for its total synthesis, in order to investigate its biological activity. 2 approaches were considered: one employing the standard scheme previously adopted for synthesizing similar compounds[4], while the second was a biogenetic-type synthetic approach. The 1st approach is still in progress, and here we would like to report on the results of the biomimetic approach.

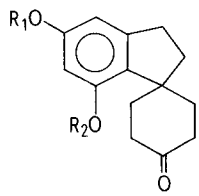
The possible biogenetic precursor of cannabispiran (**1**), namely the bibenzyl **2**, was synthesized by reacting 3-benzyloxy-5-methoxybenzaldehyde with the phosphorane obtained by reacting p-benzyloxybenzyl chloride with triphenylphosphine, in the presence of n-butyl lithium. The resulting oily stilbene (100% yield, mostly E) was hydrogenated, in the presence of 5% Pd/C at 1.03 bar, to produce **2** in a nearly quantitative yield. It was obtained as colorless prisms, m.p. 109–110°; ^{1}H NMR-spectrum (60 MHz, $CDCl_3$) showed the expected AA'BB' system for the protons of ring B at δ 6.67 and δ 7.00, J = 8 Hz. The 2 methylene groups absorbed as a 4-proton singlet at δ 2.77 and so did the 3 protons of ring A, appearing as a singlet at δ 6.23. The structure was unequivocally confirmed, however, by studying its ^{13}C NMR-spectrum (15.06 MHz, CD_3–C≡N). It exhibited 2 methylene signals at δ 38.6 and δ 37.0, 2 phenolic carbons at δ155.7 and δ158.7 (of ring A and ring B, respectively), an aromatic methoxylated carbon at δ161.8, 2 aromatic quaternary carbons at δ145.5 and δ134.0, and the unsubstituted aromatic carbons at δ 99.7, 108.9, 106.9 (ring A) and δ115 and δ130.3 (ring B).

The crucial step in the synthetic scheme involved oxidative phenol coupling of **2** to produce **3**. Several reagents were tried, unsuccessfully, like $FeCl_3$[5], $FeCl_3$-DMF complex[5], $VOCl_3$[6], VOF_3[6], $AgNO_3$[6], thallium trifluoroacetate[6] and manganese acetylacetonate[7]. However, the use of $K_3Fe(CN)_6$ in a biphasic system containing chloroform and aqueous solution of ammonia and ammonium acetate, provided the 2 dienones **3** and **4** as a 1:1 amorphous mixture (M+ at m/e 242, 2%), inseparable by tlc but was partially separated by GLC. The mixture exhibited 2 carbonyl absorption bands in the IR at $\nu_{max}^{CHCl_3}$ 1662 and 1669 cm^{-1}. Its ^{1}H-NMR-spectrum (acetone-d_6) showed in the olefinic region 2 partially overlapping AB systems of the dienone moiety and 2 methoxy signals at δ 3.57 and δ 3.70.

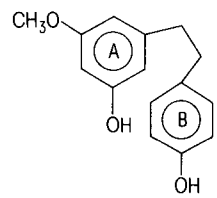
Hydrogenation of this mixture under atmospheric pressure using 5% Pd/C provided a 1:1 mixture of cannabispiran (**1**) (M+ at m/e 246[2], 34%) and its isomer **5** (M+ m/e 246, 27%) which were separated by chromatography on silica gel. The natural and synthetic compounds were indistinguishable.

Further attempts to separate the dienone mixture are being carried out. The results, along with the full characterization of **5** will be reported later. Work is also in progress on the optimization of the cyclization step. Preliminary indications showed that $MoOCl_4$ in carbon tetrachloride could accomplish this reaction in higher yields (about 35%).

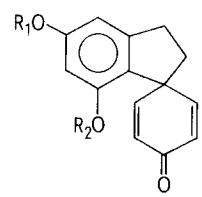
Cannabispiran (**1**) and its derivatives are some of the simplest compounds to be elaborated naturally by oxidative phenol coupling. While many reagents, notably $VOCl_3$[6] and $FeCl_3$-DMF[5] have demonstrated remarkable ability in duplicating oxidative phenol coupling in the laboratory, they failed to produce the spirosystem of cannabispiran (**1**), presumably because of the unique indan system. They were successful when the spiro system contained the less strained



1, $R_1 = CH_3$, $R = H$
5, $R_1 = H$, $R_2 = CH_3$



2



3, $R_1 = CH_3$, $R_2 = H$
4, $R_1 = H$, $R_2 = CH_3$

tetralin system. Also, in the latter system, they favored exclusively p-p-phenol coupling producing a single dienone[5,6]. Further work should shed more light on the reagents most favorable for construction of the spiro system containing an indan moiety.

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Amino acid composition and sequence of crinia-angiotensin, an angiotensin II-like endecapeptide from the skin of the Australian frog *Crinia georgiana*[1]

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Summary. Methanol extracts of the skin of the Australian amphibian *Crinia georgiana* contain large amounts of crinia-angiotensin II, a new angiotensin II-like peptide. This differs sharply from the conventional octapeptide angiotensins II in having attached the tripeptide Ala-Pro-Gly- to the N-terminus, and having an Ile residue substituted for the Val residue at position 6 from the C-terminus. Small amounts of angiotensin-like peptides have been traced, by radioimmunoassay, in skin extracts of some other *Crinia* species.

A new peptide, which can be included in the angiotensin family, has been traced in the skin of the Australian frog *Crinia georgiana.* The peptide, called crinia-angiotensin, has been isolated in a pure form and its structure identified as follows:

Ala-Pro-Gly-Asp-Arg-Ile-Tyr-Val-His-Pro-Phe

It may be seen that crinia-angiotensin strikingly differs from all other known angiotensins (ox, horse, fowl, snake angiotensins) in that it has a tripeptide (Ala-Pro-Gly-) attached to the N-terminal Asp residue of the conventional angiotensins, and in that a Ile residue is substituted for the usual Val residue at position 6 from the C-terminus.

Materials. The dried skins of 357 specimens of *Crinia georgiana,* collected in Western Australia during the period 1973–1974 were used in this study. The material weighed 26.1 g (average 0.073 g per dry skin). The skins, removed from the frogs immediately after killing and dried in the shade, were subjected to 2 successive extractions with 20 parts (w/v) of 80% methanol, each extraction lasting 3–4 days. The extracts were mixed and filtered.

Isolation procedure. An aliquot of extract corresponding to 20 g of dried skins was evaporated to dryness and the residue taken up in water plus 99% ethanol to give a final concentration of 95%. After standing, the limpid supernatant was passed through a column of 170 g alkaline alumine, which was eluted with ethanol-water mixtures of decreasing concentrations of ethanol, each of 200 ml. The peak of angiotensin-like bioactivity (guinea-pig ileum preparation) appeared in the 60% ethanol eluates. Ethanol eluates 60_2 and 60_3 were mixed and used for this study.

One-tenth of the above active eluates (= 2 g dried skin) was evaporated, the residue taken up in 4 ml distilled water adjusted to pH 3 with formic acid, and then passed through a column (9×750 mm) of SP-Sephadex (NH_4^+ form). Linear gradient elution was carried out (fractions of 5 ml) with 150 ml H_2O and 150 ml 0.5 N $HCOONH_4$ (pH 6.5). 3.5×10^{-7} moles of pure peptide were recovered in fractions 24–26.

Structure. Amino acid composition, after the usual acid hydrolysis, was as follows: His 1, Arg 1, Asp 1, Pro 2, Gly 1, Ala 1, Val 1, Ile 1, Tyr 1 and Phe 1.

2 µg of peptide, dissolved in 50 µl of 0.1 N triethylamine bicarbonate (pH 8.5), were digested at 37 °C, overnight, by adding 5 µl of TPCK-trypsin solution (1 mg/ml). The reaction mixture was dansylated. 2 dansylated fragments could be separated by TLC on silica gel, developed first with acetone and then with n-butanol : acetic acid : water (4:1:5).

After acid hydrolysis (6 N HCl, 110 °C for 24 h), the 2 fragments showed the following amino acid composition: DNS-Ala (Arg, Asp, Pro, Gly) and DNS-Ile (His, Pro, Val, Phe, O-DNS-Tyr).

Repeating the above treatment with β-chymotrypsin produced similarly 2 dansylated fragments: DNS-Ala (Arg, Asp, Pro, Gly, Ile, O-DNS-Tyr) and DNS-Val (His, Pro, Phe).

This 2nd fragment was identical with that obtainable from Val[5]-angiotensin II: DNS-Val-His-Pro-Phe.

Thus, the following partial sequence of crinia-angiotensin may be suggested:

Ala-(Asp, Pro, Gly) Arg-Ile-Tyr-Val-His-Pro-Phe

5 µg of peptide were treated with sodium in liquid ammonia as described by Araki et al.[2]. After reduction, the mixture was hydrolysed with 6 N HCl and the hydrolysate was analysed, showing lack of the Ala and His residues. This strongly suggests the presence of Ala-Pro and His-Pro bonds in the sequence.

The dansyl Edman procedure was then performed on 10 nmoles of the intact peptide, obtaining the sequence

Ala-Pro-Gly-Asx-Arg-Ile-Tyr-Val-His-Pro-Phe

In the last step of degradation, dansyl Phe was detected without acid hydrolysis. This suggests that the C-terminal position of the peptide was not blocked.