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CARBONYL INSERTION REACTION ON CUPRIC ACETOACETIC ETHYL ESTER USING CARBONYL SULPHIDE

THE reaction of cupric acetoacetate ethyl ester with phosgene was reported to give 2 : 6 dimethyl 3 : 5 dicarbethoxy 1 : 4 pyrone.[1] We now report that the same reaction can be achieved by using carbonyl sulphide in place of phosgene. The former can be generated by the action of dil. H_2SO_4 on potassium ethyl thiocarbonate (Bender's Salt).

EXPERIMENTAL

A solution of 0·025 mole of cupric acetoacetic ethyl ester in toluene (40 ml.) was taken and added to toluene containing excess of dissolved carbonyl sulphide. The tightly stoppered bottle containing the mixture was allowed to stand for about 48 hours with occasional shaking. The green colour due to the copper chelate gradually disappeared and a black precipitate of cupric sulphide was thrown out. The solution was filtered and then distilled. The residual viscous liquid on standing gave a very small amount of crystalline substance. This on recrystallization from benzene gave a pure compound with m.p. 80° C. Analysis C = 58·14; H = 6·05; $C_{13}H_{16}O_6$ requires C = 58·2; H = 6·012.

The mixed melting point with an authentic sample of 2 : 6 dimethyl 3 : 5 dicarbethoxy 1 : 4 pyrone was undepressed. The very poor yield of the same may be due to incomplete cyclisation of the compound under the experimental conditions.

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INDUCTION OF DIPLOIDS IN FORCED HETEROCARYONS OF *ASPERGILLUS NIGER* VAN TIEGHEM

FUSION of two haploid nuclei produces a diploid. Attempts were made to induce diploids in forced heterocaryons of *A. niger* (Table I). These heterocaryons grew well and conidiated adequately on minimal medium[1] except HC-5 which grew but produced no conidia. They were incubated for a week at 25° C. on minimal medium with and without the treatment of diploid inducing agents.[2] Those presumed to be diploids in these experiments were those recovered and tested, by single spore culture, by measurement of conidial size, and by treatment of conidia with *p*-flurophenyl-alanine.

The results showed that heterocaryon HC-3 and HC-4 produced diploids spontaneously whereas HC-1 and HC-5 required the treatment of a diploid inducing agent while HC-2 failed to produce diploid. HC-5 which did not conidiate in the original stage conidiated abundantly in the diploid stage. The single spore culture from each diploid appeared stable and similar to the wild type, except the HC-5 diploids. The latter culture was yellow rather than black indicating homozygosity for the colour marker. It was suggested by Roper (1952) that usually the size of diploid conidia was 1·3 times larger in diameter than the mean size of the parental conidia. This was (Table I) for the diploid of HC-3 and HC-4 whereas in HC-1 and HC-5 the ratio was 1 : 1·18, 1 : 1·08, respectively. When all the diploids were treated with *p*-flurophenylaline, they segregated into their parental components, thus proved that they were in fact true diploids.

These results suggest that diploid inducing agents may have very little effect in inducing diploids. Roper (1966) also reported that camphor vapour did not induce diploids in *Aspergillus* but rather selected out a higher proportion of natural diploid nuclei. It is suggested that when two dissimilar nuclei in heterocaryon divide synchronously they have greater opportunity to fuse and thereby form a diploid as is probable in case of HC-1, HC-3, HC-4, and HC-5. However, if they fail to divide synchronously it is difficult to produce diploid as is the case with HC-2.

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TABLE I

Conidial size of parent heterocaryons and induced diploids in Aspergillus niger *and their comparative ratios*

Heterocaryons No.	Symbol of Mutants	Conidial size of parents (μ)	Conidial size of diploids (μ)	Ratio of diploid conidial size to average of parental conidial size (μ)
HC-1	gl paba × yl hypox	3·27×3·40 (3·33)*	3·92	1 : 1·18
HC-2	gl paba × yl hist	3·27×3·45 (3·36)	..	..
HC-3	gl meth × yl hypox	3·31×3·40 (3·35)	4·45	1 : 1·35
HC-4	gl meth × yl hist	3·31×3·45 (3·38)	4·57	1 : 1·35
HC-5	yl hypox × yl hist	3·40×3·45 (3·42)	3·72	1 : 1·08

* Average of both parental conidial size.

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ISOLATION OF LUTEOLIN AND GLUCOLUTEOLIN FROM THE FLOWERS OF *TRIDAX PROCUMBENS*

Tridax procumbens Linn. (Family : *Compositæ* is a straggling herb bearing small yellow flowers found all over South India. Fresh flowers were collected during December-January, extracted thrice with methanol[1,2] by cold maceration and the total extract was concentrated *in vacuo* to a small volume. It was shaken with petroleum ether followed by ether. The ether extract on concentration yielded a yellow crystalline solid which after recrystallisation from methanol came out as yellow needles, m.p. 318–20. This was identified as luteolin by its R_f values on paper chromatography and preparation of its acetate, m.p. and mixed m.p. 224–26°. It was also compared with an authentic sample of luteolin. The ether extract left after the separation of luteolin indicated another spot on chromatography which had the R_f values of quercetin.

The aqueous layer on keeping in an ice-chest for 2 weeks deposited some light yellow crystalline solid which on recrystallisation from methanol-ether yielded pale yellow needles, m.p. 240–42° which was undepressed on admixture with an authentic sample of glucoluteolin.[3] The glycoside on acid hydrolysis yielded luteolin and glucose. Hence it was identified as glucoluteolin. The mother liquor after removal of glucoluteolin was studied by paper chromatography and isoquerecetin could be identified by comparison of R_f values with those of an authentic sample.

A portion of the mother liquor was hydrolyzed with 7% H_2SO_4 to yield quercetin, luteolin and glucose. The total yield of the pigment mixture was about 0·5% on the dry basis with a predominance of free luteolin.

The occurrence of luteolin and its 7-glucoside together with quercetin in *T. procumbens* is in agreement with earlier record on the distribution of flavonoids in the *Compositæ*.[4]

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ON THE OCCURRENCE OF A HORN-CORE OF A BOVID IN THE RAJAHMUNDRY SANDSTONES OF PANGADI, A.P.*

THE present communication records the discovery of a portion of a horn-core of a bovid, presumably the first record of a vertebrate fossil, in the Rajahmundry sandstones at Chagallu (16° 59′ ; 81° 40′ ; 65 H/9), about 4 km., south of Pangadi, West Godavari District, Andhra Pradesh. A brief description of the horn-core is given below :