surface of the capsule is granular owing to the sand grains that adhere to it. (Compare Van Cleave's description of *Pleurocera* eggs.)

No data are available on the duration of embryonic development for either species, though it is probably not widely different from the 11½ days reported by Jewell. Figure 3 shows a young specimen of *G. virginica* hatched in the laboratory; Figure 4 shows a young *A. carinata* found in the river; unfortunately none of the eggs brought in hatched.

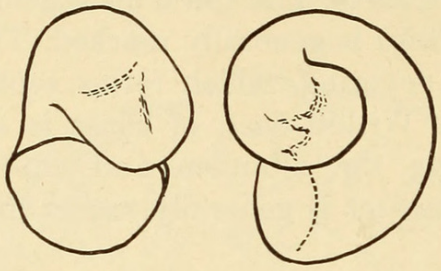

Fig. 4.—Young shell of *Anculosa carinata*. ×72.

All of the drawings in this paper were made by the staff artist of this department, Mr. Arthur Johansen, with a camera lucida.

CHEMISTRY.—*The rotenone content of derris root, cube root, and other plant materials.*[1] HOWARD A. JONES, Bureau of Chemistry and Soils. (Communicated by C. M. SMITH.)

Rotenone, a constituent of derris root (*Deguelia* sp.) and of cube root (*Lonchocarpus nicou*), has recently come into prominence as an insecticide of considerable value. An extraction method[2] making use of carbon tetrachloride was recently outlined by the author for the determination of this compound in plant materials. The present article gives the results obtained by this method, and by an ether extraction method previously in use, in the analysis of plants of the genus *Deguelia* from the Malay Peninsula and the East Indies, and plants of the genus *Lonchocarpus* from South America.

Rotenone has been previously reported as occurring in species of both of these genera of plants. Nagai[3] was the first to isolate rotenone from a species of *Deguelia*, the roots of *D. chinensis*. By ether extraction of the roots of *D. elliptica*, Kariyone and Atsumi[4] obtained 6.65

[1] Received October 12, 1932.

[2] Ind. Eng. Chem., Anal. Ed., vol. 5, no. 1, Jan. 15, 1933.

[3] Jour. Tokyo Chem. Soc., vol. 23, p. 744, 1902.

[4] Jour. Pharm. Soc., Japan, no. 491, p. 10, 1923.

per cent crude rotenone; while Takei,[5] also using ether, found the rotenone content of several samples of roots of the same species to range from 1.5 to 6 per cent. Recently Spoon[6] has analyzed a number of samples of roots of species of *Deguelia* by the ether extraction method outlined by Roark.[7] He found that *D. elliptica* ranged in rotenone content from traces up to 6 per cent, and *D. malaccensis* contained from traces up to 2 per cent. A number of commercial samples of derris root of unknown species he found contained from no rotenone up to 6.5 per cent.

The rotenone content of stems of *Lonchocarpus nicou* from French Guiana was reported as 2 to 2.5 per cent by Geoffroy,[8] who used a petroleum ether extraction, as early as 1895. Clark,[9] by ether extraction, recently obtained 7.1 and 7.2 per cent rotenone from two samples of cube root (*L. nicou*) from Peru.

Spoon[10] has also determined the amount of rotenone in both the stems and roots of nekoe (an unidentified species of *Lonchocarpus* from Dutch Guiana) by the ether extraction method used for derris roots. He found that the root averaged about 2.5 per cent rotenone, while a sample of stem material contained only 0.03 per cent. Rotenone has been reported by Tattersfield, Gimingham and Morris[11] as occurring in haiari stems and roots (a species of *Lonchocarpus* found in British Guiana) "in fairly considerable quantities." Pfaff[12] has reported "timboin," which appears to have been impure rotenone, in Brazilian timbo root (probably a species of *Lonchocarpus*).

One of the objects of this investigation was to obtain a comparison of the ether extraction method, previously in use by the Insecticide Division of this Bureau, with the carbon tetrachloride method recently developed. As a result of the analyses reported in this paper the latter method has been adopted. A further purpose of the present work was to determine the variation in the rotenone content of derris root, cube root and other plant materials, and to ascertain the desirability of these materials as sources of supply of rotenone.

Of the samples of derris root tested, three were authentic speci-

[5] Bul. Inst. Phys. Chem. Research (Tokyo), vol. 2, p. 485, 1923.

[6] Indische Mercuur, vol. 54, no. 18, p. 351, 1931, and vol. 55, no. 13, p. 181, 1932.

[7] Soap, vol. 7, p. 97, 1931.

[8] Ann. Inst. Colon. Marseille, vol. 2, p. 1, 1895.

[9] Science, vol. 70, p. 478, 1929.

[10] Indische Mercuur, vol. 54, no. 49, p. 1043, 1931.

[11] Ann. Appl. Biol., vol. 13, p. 424, 1926.

[12] Arch. der Pharmacie, vol. 229, p. 31, 1891.

mens of *D. elliptica* and two were authentic *D. malaccensis*.[13] Unfortunately the remaining samples analyzed were received merely under the name of "derris root" or "tuba root" and were of unknown species. In many cases the original source was also unknown. Since the majority of the root in commerce is said to be *D. elliptica*, it is probable that many of the samples bought in the open market were of this species. All samples of derris root were found to conform in histological elements[14] to an authentic sample of *D. elliptica* and hence are almost certainly of the genus *Deguelia*.

One sample of authentic *L. nicou* was obtained (under the name of "barbasco" or "cube") from the Field Museum of Natural History in Chicago. Other specimens of cube and barbasco roots were compared with this sample and found to be identical in histological elements. All samples of cube or barbasco roots were obtained from Peru and, according to Killip and Smith,[15] these native terms are restricted, in that region, to *L. nicou*.

A sample of timbo root obtained from Brazil was stated to be *Paullinia pinnata*, but from the findings of Killip and Smith, it is more likely that this material is a species of *Lonchocarpus*. This is borne out by the fact that this sample closely corresponded in histological elements to an authentic sample of *L. nicou*. One sample of nekoe stems from Dutch Guiana and two samples of haiari stems from British Guiana were analyzed.

The results of the extractions are given in the tables, in which the samples have been divided into three groups; roots of the genus *Deguelia*, roots of *L. nicou*, and samples of other plants of the genus *Lonchocarpus*. The numbers are Insecticide Division sample numbers and are given merely for convenience in referring to the samples. Values for both rotenone and total extractive material are given for both methods. (In a number of cases the sample was analyzed by only one method.) In the majority of cases the results given represent the mean of two or more determinations. All results are based on the weight of air-dried material, the moisture content of all samples at the time of analysis being between 2 and 5 per cent. A few samples received in a more moist condition than this were dried to within this

[13] Two samples of *D. elliptica* roots and two of *D. malaccensis* roots were obtained through the courtesy of the Department of Agriculture of the Federated Malay States and Straits Settlements.

[14] The cooperation of George L. Keenan of the Food and Drug Administration, U. S. Department of Agriculture in making microscopic examinations is gratefully acknowledged.

[15] This JOURNAL, vol. 20, p. 74, 1930.

range before grinding. All roots not otherwise designated in Table I were bought on the open market in this country and were of unknown original source.

TABLE I. ROOTS OF DEGUELIA SPECIES (DERRIS ROOT)

Sample Number	Carbon Tetrachloride Method		Ether Method		Remarks
	Rotenone (per cent of air-dried material)	Total Extract (per cent of air-dried material)	Rotenone (per cent of air-dried material)	Total Extract (per cent of air-dried material)	
401	None	9.4	None	9.8	Included some stem and leaf parts. Bought in Holland.
402	1.4	7.6	1.0	8.2	
406	None	16.3	None	14.8	Java, Dutch East Indies.
407			3.0	16.0	
408	2.0	19.6	None[a]	20.0	
411	0.9	9.4			
412	None	22.6	None[b]	19.6	
502			0.8	5.4	
522	6.7	22.2	6.8	21.6	Shipped from Singapore.
523	1.4	13.6	1.4	12.2	*D. elliptica* (Tuba Puteh) Malayan Govt. Exp. Plantn., Serdang, F.M.S.
524	1.8	21.0	None[a]	22.6	*D. malaccensis* (Tuba standing) Malayan Govt. Exp. Plantn., Serdang, F.M.S.
535	4.5	20.7	4.3	17.9	
537	1.9	20.8	None[a]	23.4	Dutch East Indies.
547			About 0.4	14.3	Federated Malay States.

[a] No rotenone separated even when seed crystals were used.

[b] 2 to 3 per cent amorphous material separated from the ether extract, but no rotenone was detected in this.

TABLE I. (*Continued*).

Sample Number	Carbon Tetrachloride Method		Ether Method		Remarks
	Rotenone (per cent of air-dried material)	Total Extract (per cent of air-dried material)	Rotenone (per cent of air-dried material)	Total Extract (per cent of air-dried material)	
548	About 0.1	20.6	—[c]	19.2	Sumatra, Dutch East Indies.
549	1.9	17.8	2.0	16.8	Federated Malay States.
550			0.6	13.0	
585	None	—[d]	None[e]	12.1	Bought in Holland.
586			1.5	16.2	
588-A	0.6	11.9	About 0.3	10.0	
588-B			2.2	9.9	
588-C			3.2	15.9	
588-D			3.0	12.2	
588-E	1.0	11.4	About 0.4	9.4	
588-F			1.9	10.4	
(A) coarse 594(B) fine Aver. whole root[f]	3.4 6.0 5.2	12.3 19.1 17.1	3.0 5.9	12.4 18.9	Shipped from Singapore.
611			1.6	15.9	Shipped from Singapore.
612			1.7	9.5	Shipped from Singapore.
621	2.4	19.2	1.0	18.2	
626			0.9	16.3	

[c] About 2 per cent material separated from the ether extract; this was found to contain only a very small proportion of rotenone.

[d] Total carbon tetrachloride extract not determined.

[e] About 0.6 per cent material separated from the ether extract, but no rotenone was detected in this.

[f] This average is based on the proportion of fine and coarse roots comprising the whole root sample.

TABLE I. (*Continued*)

Sample Number	Carbon Tetrachloride Method		Ether Method		Remarks
	Rotenone (per cent of air-dried material)	Total Extract (per cent of air-dried material)	Rotenone (per cent of air-dried material)	Total Extract (per cent of air-dried material)	
685			3.0	14.6	
739	1.7	15.6	None[a]	14.6	Shipped from Singapore.
741	1.8	9.3	1.3	8.8	*D. elliptica* Malayan Govt. Exp. Plantn., Serdang, F.M.S.
742	2.1	24.0	1.2	24.7	*D. malaccensis* Malayan Govt. Exp. Plantn., Serdang, F.M.S.
(A) coarse 743(B) fine Aver. whole root[f]	2.0 2.3 2.1	15.5 16.3 15.8	0.3 0.4	15.2 17.1	*D. elliptica* F.M.S.
765	6.3	21.4	5.5	20.2	
875	2.9	12.0			Shipped from Singapore.
956	6.9	21.4			
998	None	15.4			Sumatra, Dutch East Indies.
999	None	13.7			Sumatra, Dutch East Indies.
1000	None	16.5			Sumatra, Dutch East Indies.
1001	5.7	16.8			Shipped from Singapore.
1002	5.3	16.7			Shipped from Singapore.

[a] No rotenone separated even when seed crystals were used.

[b] This average is based on the proportion of fine and coarse roots comprising the whole root sample.

TABLE I. (*Continued*)

Sample Number	Carbon Tetrachloride Method		Ether Method		Remarks
	Rotenone (per cent of air-dried material)	Total Extract (per cent of air-dried material)	Rotenone (per cent of air-dried material)	Total Extract (per cent of air-dried material)	
1003	6.1	17.4			Federated Malay States.
1004	4.2	15.1			Federated Malay States.
Average[g]	2.5	16.5			

[g] This average is for 31 samples analyzed by the carbon tetrachloride method. No. 585 is not included in the average because no determination of total extract was made on this sample. The average values for whole root of Nos. 594 and 743 were used in obtaining the final average.

TABLE II. Roots of Lonchocarpus nicou (cube root)

Sample Number	Carbon Tetrachloride Method		Ether Method		Remarks
	Rotenone (per cent of air-dried material)	Total Extract (per cent of air-dried material)	Rotenone (per cent of air-dried material)	Total Extract (per cent of air-dried material)	
425			1.4	12.2	
426	0.8	6.1	None[a]	4.1	
427	1.8	12.0	1.4	11.1	
433	6.8	20.0			"Barbasco or Cube" from Field Museum of Natural History, Chicago. Authentic *L. nicou.*
527	1.3	9.2	1.6	9.5	
583	6.1	16.5	6.1	16.0	
584	6.0	18.5	6.1	17.6	
674	1.5	13.8	2.0	13.7	
686-A	11.2	24.3	11.0	23.8	
686-B	6.7	14.8			
686-C	4.0	12.2	5.0	10.8	
686-D	8.5	18.2			

[a] No rotenone separated even when seed crystals were used.

TABLE II. (*Continued*)

Sample Number	Carbon Tetrachloride Method		Ether Method		Remarks
	Rotenone (per cent of air-dried material)	Total Extract (per cent of air-dried material)	Rotenone (per cent of air-dried material)	Total Extract (per cent of air-dried material)	
686-E	7.4	17.2	7.2	15.9	
686-F	5.5	13.4			
686-G	6.3	15.0	6.3	13.8	
686-H	5.6	12.9			
686-I	6.1	14.3	5.8	14.4	
740	3.9	13.3			
821	4.1	16.7	3.6	15.6	
867	3.4	12.9			
940-A	8.6	20.2			
940-B	6.1	13.7			
1025	8.0	22.4			Received as "Barbasco Root."
Average[b]	5.4	15.3			

[b] This average is for the 22 samples analyzed by the carbon tetrachloride method.

TABLE III. OTHER PLANTS OF THE GENUS LONCHOCARPUS

Sample Number	Plant Material and Source	Carbon Tetrachloride Method		Ether Method	
		Rotenone (per cent of air-dried material)	Total Extract (per cent of air-dried material)	Rotenone (per cent of air-dried material)	Total Extract (per cent of air-dried material)
409	Timbo Roots—Brazil	About 5[a]	28.0		
434	Haiari stems	None	2.6	None	2.8
627	Haiari stems—British Guiana	1.0	6.2	None[b]	6.6
990	Nekoe stems—Dutch Guiana	About 0.4	3.5		

[a] 6 to 7 per cent material separated, but this was found to be only partly rotenone; value given for rotenone estimated from examination of separated material.

[b] No rotenone separated even when seed crystals were used.

Some of the advantages of the carbon tetrachloride extraction method over the method using ether are clearly illustrated by the results in the tables. For instance, in the cases of derris root samples 408, 524, 537 and 739, cube root sample 426 and haiari stem sample 627, no rotenone was obtained by the ether method even when the evaporated extract was seeded with rotenone crystals. On the other hand, between about 1 and 2 per cent rotenone was obtained by the carbon tetrachloride extraction of these same samples. Decidedly lower values for rotenone were obtained by the ether method in the cases of derris roots 588-E, 621, 742 and 743. Furthermore, the ether extracts of three derris root samples (412, 548 and 585) yielded varying amounts of material which was found to contain either no rotenone, or, in the case of 548, only a small proportion of rotenone. The carbon tetrachloride extracts of these three samples gave no separation of material, or, in the case of 548, only about 0.1 per cent rotenone. The ether extraction values for rotenone given in the tables are therefore not reliable.

Perhaps the most striking observation to be made is the wide range in the rotenone content of both derris and cube roots. Thus the rotenone in derris root ranges from none to almost 7 per cent, and that in cube (or barbasco) root from less than one to about 11 per cent. This variation is probably due in part to differences in age, size of roots, soil conditions and other factors. In the case of the derris root samples the variation is no doubt partly due to differences in species.

An examination of the values for rotenone and total extract of derris root shows that there is little or no correlation between either the total carbon tetrachloride extractives or the total ether extractives and the rotenone content. Good correlation could hardly be expected since the samples are of different species and from different sources. However, there is a comparatively close correlation between the total carbon tetrachloride extractives and rotenone in the cube root samples, the coefficient of correlation being 0.86. Such a correlation is, no doubt, due to the fact that the cube samples are all of the same species (*L. nicou*) and all from the same region. The values for total ether extract and rotenone content of cube roots also show a fairly good correlation. However, the calculation of the rotenone content of a sample of cube root from the amount of total extract would give only approximate results.

As will be noted, the two authentic samples of *D. malaccensis* tested contained only about 2 per cent rotenone, in spite of their high total

extract values. This agrees with the results of Spoon (loc. cit.) on this species. It is possible that some of the other samples of derris root having high total extracts were also of this species.

According to Georgi[16] who has made a study of the proportion of ether extractives obtained from coarse and fine roots of *D. elliptica* and *D. malaccensis* of different ages, the ether extract content of fine roots is higher than that of coarse roots. It was thought of interest to determine whether this was also true of the rotenone content. Accordingly two samples of derris root (594 and 743) were separated into fine and coarse roots and analyzed separately. Roots smaller than 4.0 to 4.5 mm. in diameter were arbitrarily considered "fine" and those of this size and over were designated as "coarse." The fine roots constituted about 71 per cent of the whole root in the case of No. 594 and about 32 per cent in the case of No. 743. It will be seen from Table I that in both samples the rotenone content (by the carbon tetrachloride method) of the fine roots was higher than that of the coarse roots.[17] This was also true of the total extractive materials.

Some idea of the variation to be expected in single shipments of both derris and cube roots is afforded by the results in the table.Thus samples 588-A to 588-F, inclusive, were taken from six 80 to 100-pound bales of a single shipment of derris root. As will be noted the rotenone content ranges from a few tenths of one per cent to about 3 per cent. Similarly samples 686-A to 686-E were taken from five 65 to 100-pound bales of a single shipment of cube root. The lowest rotenone content found in this shipment was 4 per cent while the highest was about 11 per cent. Samples 686-F to 686-I are from four bales of a second consignment of this shipment. Samples 940-A and 940-B of cube root were also from a single shipment. This variation in single shipments should emphasize the necessity of careful sampling of shipments of root for analysis.

The high rotenone content of the timbo root tested indicates that this material merits further study as a possible source of rotenone. The low values for total extractives obtained from haiari and nekoe stems are interesting. Spoon (loc. cit.) obtained about 2.5 per cent ether extractives from nekoe stem, which indicates that these materials are consistently low in total extract.

A more systematic study of the rotenone content of authentic samples of the numerous species of *Deguelia* and *Lonchocarpus* should

[16] Malay. Agr. Jour., vol. 17, p. 326, 1929, and vol. 17, p. 361, 1929.

[17] The difference in rotenone values in sample 743 is perhaps too small to be of much significance.

be made by those in a position to obtain such material. The stem parts of both derris and cube should be investigated for their rotenone content as it is possible that these materials may contain sufficient rotenone to justify harvesting the whole plant rather than only the roots.

CONCLUSIONS

1. The rotenone content of 45 samples of derris root tested ranged from none to about 7 per cent, while that of 23 samples of cube root ranged from less than one to about 11 per cent. The average of the 31 samples of derris root analyzed by the carbon tetrachloride method was 2.5 per cent rotenone, while the average for the 22 samples of cube root analyzed by this method was 5.4 per cent rotenone. These averages should not be considered representative of derris and cube samples in general.

2. These results indicate the desirability of cube root as a source of rotenone. More extensive cultivation of this material is indicated. By selection of high rotenone strains of derris, the rotenone content of this plant could, no doubt, be improved.

3. A close correlation exists between the rotenone content and the total extractive materials of cube root. There is little or no correlation between these two values in the case of the derris root samples.

4. Fine derris roots have a slightly higher rotenone content than coarse roots.

5. Brazilian timbo root may afford an additional source of rotenone.

PALEONTOLOGY.—*Unique coloration of two Mississippian brachiopods.* R. R. Rowley, Louisiana, Mo., and James S. Williams, U. S. Geol. Survey. (Communicated by John B. Reeside Jr.)

New and striking types of color patterns on fossils are always interesting and worthy of record, even though their origin may not be fully understood. Such a pattern has recently been discovered on two specimens of *Acanthospirina aciculifera* (Rowley) Schuchert and LeVene, and because it is apparently unique and may possibly be original, and because, even if not original, it may serve to focus critical attention on other color patterns that have been described as original, it seems advisable to call attention to it.

The color-marked specimens were collected by R. R. Rowley from the yellow-brown shale at the base of the Louisiana limestone (Lower

Jones, Howard Allen. 1933. "The rotenone content of derris root, cube root, and other plant materials." *Journal of the Washington Academy of Sciences* 23, 36–46.

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